LEAF BLACKENING OF PROTEAS

BY

Iain Andrew Stephens

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PROMOTER

Prof. G. Jacobs - Department of Horticultural Science, University of Stellenbosch

CO-PROMOTER

Dr. D.M. Holcroft - Department of Horticulture, Michigan State University

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DECLARATION

I, the undersigned hereby declare that the work contained in this dissertation is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

Signature

Date

SUMMARY

Leaf blackening is a particular problem limiting vase life and marketability of *Protea* cut flowers. This research investigated suppression of *Protea* leaf blackening with a specific focus on *Protea* cv. Sylvia (*P. eximia* × *P. susannae*) cut flowers.

Leaf blackening decreased significantly with decreasing storage temperatures in 'Sylvia' proteas and this was attributed to lower respiration rate and conservation of carbohydrate. Low storage temperatures were beneficial in short term handling procedures encountered during airfreight. However, use of low temperatures alone during the longer sea freight period was unsatisfactory in either maintaining or extending 'Sylvia' protea vase life. Cooling of 'Sylvia' proteas under vacuum significantly suppressed leaf blackening and was of greater benefit than forced air cooling.

Although removal of the uppermost leaves delayed leaf blackening in short term storage no significant benefit was found for longer storage periods. Girdling directly beneath the 'Sylvia' protea flowerhead significantly reduced leaf blackening and in combination with low storage temperatures (0°C) enabled a significant extension in both storage and vase life of 'Sylvia' proteas.

'Sylvia' proteas did not exhibit a climacteric respiration peak during 96 h storage at 0°C. Exposure to ethylene did not increase *Protea* leaf blackening or have a detrimental effect on vase life of either proteas or pincushions evaluated.

No beneficial response to sucrose supplementation was found in 'Sylvia' proteas. Analysis of the sugar content of both flowerhead and leaves indicated that glucose

supplementation might be of benefit and was investigated. Holding solutions of 2.5% glucose significantly extended vase life due to a significant reduction in leaf blackening. Vase life was terminated due to flowerhead collapse instead of leaf blackening for the first time in 'Sylvia' protea cut flowers. Vase life was significantly extended by ≥3% glucose pulse solutions and leaf blackening significantly suppressed with increasing glucose pulse concentration. Solution uptake was facilitated by use of high intensity PAR lights in the early morning and was attributed to increased stomata opening and a consequent increase in both transpiration and glucose solution uptake. The faster uptake of glucose solutions in shoots harvested in the afternoon was attributed to higher shoot temperatures and consequent transpiration rate to those harvested in the morning. There was a significant reduction in uptake time with increasing pulse temperature, which enabled vacuum cooling to be performed earlier further benefiting storage and vase life extension.

Enclosure of 'Sylvia' proteas in polyethylene (PE) lined cartons did suppress leaf blackening in non-pulsed shoots. However, this had no practical significance on useful vase life, which was terminated at this point due to excessive leaf blackening. Water loss appears to have a minimal influence on 'Sylvia' protea leaf blackening.

Shading at four and three weeks prior to harvest coincided with a period of significant flowerhead dry mass increase. It is thought that shading at this point, concurrent with an increased carbohydrate demand by the developing flowerhead resulted in a temporary limitation in carbohydrate supply resulting in the appearance of preharvest leaf blackening. It would appear that proteas do not store large quantities of

carbohydrate. Although accentuating winter light conditions by shading did result in a decrease in carbohydrate content the fact that carbohydrate content was already low precluded shading from having a significant impact on postharvest leaf blackening.

The finding that glucose was beneficial in extension of both storage and vase life of 'Sylvia' proteas directed research into its use for other *Protea* and *Leucospermum* cut flowers. Significant differences in the response to glucose supplementation were found in both *Protea* and *Leucospermum* (pincushions). The significant difference in sensitivity to glucose concentration in 'Pink Ice' proteas (phytotoxic at ≥4%) and 'Susara' proteas (no apparent toxicity), in conjunction with a lack of response in 'Cardinal' proteas, a hybrid from the same parents as 'Sylvia' indicates the need to direct future research to individual cultivars. Glucose supplementation had no beneficial effect on vase life of 'Scarlet Ribbon' and 'Tango' pincushions, whilst significantly extending vase life of 'Cordi', 'Gold Dust', 'High Gold' and 'Succession' pincushions.

Blaarverswarting van Proteas

Opsomming

Blaarverswarting is 'n spesifieke probleem wat die vaasleeftyd en die bemarkbaarheid van *Protea* snyblomme beperk. In hierdie navorsing is ondersoek ingestel na die onderdrukking van *Protea* blaarverswarting met spesifieke fokus op die snyblomme van die kv. Sylvia (*P. eximia* × *P. susannae*).

Die voorkoms van blaarverswarting by 'Sylvia' het merkbaar afgeneem tydens die verlaging van bergingstemperature. Hierdie afname is toegeskryf aan 'n laer respirasietempo en die behoud van koolhidrate. Lae bergingstemperature in die korttermyn hantering van die produk tydens lugvrag was voordelig. Die gebruik van lae temperature, slegs tydens die langer verskeepingsperiode, was egter onbevredigend vir vaasleeftyd verlenging en onderhoud van 'Sylvia' protea. Die afkoeling van 'Sylvia' proteas onder vakuum het blaarverswarting in 'n groot mate onderdruk en het beter resultate gelewer as geforseerde lugverkoeling.

Alhoewel die verwydering van die heel boonste blare blaarverswarting by korttermynopberging vertraag het, het dit geen merkbare voordele vir langer bergingsperiodes ingehou nie. Ringelering direk onder die blomkop van die 'Sylvia' protea het blaarverswarting aansienlik verminder, en saam met lae bergingstemperature (0°C) het dit 'n merkbare verlenging in beide die bergingstyd en die vaasleeftyd van 'Sylvia' proteas teweeggebring.

'Sylvia' proteas het geen klimakteriese respirasiekruin tydens 'n bergingsperiode van 96 uur teen 0°C getoon nie. Blootstelling aan etileen het nie die *Protea* blaarverswarting laat toeneem of 'n nadelige effek op die vaasleeftyd van die proteas of speldekussings wat geëvalueer is, gehad nie.

Geen voordelige reaksie op sukrose-byvoeging is in 'Sylvia' proteas gevind nie. 'n Analise van die suikerinhoud van beide die blomkoppe en die blare het aangetoon dat 'n glukose-byvoeging moontlik voordelig kon wees, en hierdie aspek is ondersoek. Met stooroplossings van 2,5% glukose is die vaasleeftyd aansienlik verleng omdat daar 'n merkbare afname in blaarverswarting was. Vir die eerste keer in die geval van die 'Sylvia' protea, het die vaasleeftyd van die snyblomme tot 'n einde gekom omdat die blomkoppe uitmekaar gebreek het en nie omdat blaarverswarting ingetree het nie. Die vaasleeftyd is aansienlik verleng met ≥ 3% glukose-pulsoplossings, en blaarverswarting is merkbaar onderdruk met die verhoging van hierdie oplossings se glukosekonsentrasie. Die opname van die oplossings is gefasiliteer deur hoë intensiteit PAR (fotosinteties-aktiewe radiasie) ligte vroeg in die oggend, en is toegeskryf daaraan dat meer huidmondjies oopgegaan het. Dit het gelei tot 'n toename in transpirasie en 'n toename in die opname van die glukose-oplossing. Die feit dat glukose-oplossings vinniger opgeneem is deur lote wat in die middag geoes is, is toegeskryf daaraan dat loottemperature dan hoër is as soggens en gevolglik lei tot 'n vinniger transpirasietempo. Daar was 'n merkbare afname in die opnametyd wanneer die temperatuur van die pulsoplossings verhoog is. Vakuumafkoeling kon dus vroeër toegepas word, wat 'n verlenging in bergingstyd en vaasleeftyd tot gevolg gehad het.

Verpakking van 'Sylvia' proteas in kartonne wat met poli-etileen uitgevoer is, het blaarverswarting van lote wat nie aan pulsering onderwerp is nie, onderdruk. Hierdie maatreël het egter geen praktiese waarde met betrekking tot vaasleeftyd nie; die vaasleeftyd het tot 'n einde gekom as gevolg van omvangryke blaarverswarting. Dit lyk asof waterverlies weinig invloed het op die blaarverswarting van 'Sylvia' proteas.

Die vermoede bestaan dat lae koolhidraatvlakke proteas ontvanklik maak vir blaarverswarting. Alhoewel die beklemtoning van winterligtoestande deur beskaduwing gelei het tot 'n afname in koolhidraatinhoud, was hierdie inhoud reeds laag en blaarverswarting na die oes is nie beïnvloed nie. Beskaduwing tydens die vier en drie weke voor oestyd het saamgeval met 'n tydperk van aansienlike toename in die droë massa van die blomkop. Die vermoede bestaan dat beskaduwing tydens hierdie fase, saam met die toename in die ontwikkelende blomkop se behoefte aan koolhidrate, aanleiding gegee het tot 'n tydelike beperking in koolhidraatvoorraad wat die voorkoms van blaarverswarting voor die oes tot gevolg gehad het.

Die bevinding dat glukose voordelig is vir die verlenging van beide die bergingstyd en die vaasleeftyd van 'Sylvia' proteas het die navorsing gerig om ook ondersoek in te stel na die gebruik daarvan vir ander *Protea* en *Leucospermum* snyblomme. Merkbare veranderinge is gevind in die reaksie op glukosebyvoegings in beide *Protea* en *Leucospermum* (speldekussings). Die opmerklike verskil in sensitiwiteit vir glukosekonsentrasie in 'Pink Ice' proteas (fitotoksies by $\geq 4\%$) en 'Susara' proteas (geen klaarblyklike toksisiteit), saam met 'n gebrek aan reaksie by 'Cardinal' proteas, 'n hibried van dieselfde ouers as 'Sylvia', dui aan dat verdere

navorsing op individuele kultivars toegespits sal moet word. Glukosebyvoegings het geen voordelige uitwerking op die vaasleeftyd van 'Scarlet Ribbon' en 'Tango' speldekussings gehad nie, terwyl dit die vaasleeftyd van 'Cordi', 'Gold Dust', 'High Gold' en 'Succession' speldekussingkultivars merkbaar verleng het.

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1. INTRODUCTION AND LITERATURE REVIEW - Leaf blackening in *Protea* cut flowers

Leaf blackening in *Protea* cut flowers

Introduction

Several South African *Protea* species have been selected for commercial propagation on the basis of an attractive inflorescence. The inflorescence is defined as "an involucrate capitulum, consisting of multiple individual flowers arising from inside the involucral bracts" (Johnson & Briggs, 1975). South African production of *Protea* cut flowers has undergone rapid development in the last decade with increased commercial production and a move away from the harvesting of natural stands. A number of commercial species and hybrids formed from them, are particularly susceptible to leaf blackening, for example, *P. compacta* (Haasbroek *et al.*, 1973), *P.* cv. Pink Ice (*P. susannae* × *P. compacta*) (McConchie *et al.*, 1991), *P. eximia* (Paull *et al.*, 1980), *P. neriifolia* (de Swardt, 1979), *P.* cv. Pink Mink (*P. neriifolia*) (Dai & Paull, 1995) and *P. magnifica* (de Swardt *et al.,* 1987).

SAPPEX export figures of *Protea* cv. Sylvia, a selection made from a *P. eximia* (Salisb. ex Knight) Fourc. × *P. susannae* E. Phillips crossing, indicate a rapid growth in 'Sylvia' protea production and export (Table 1) (SAPPEX, 1995,1999-2002). This increase may be attributed in part to the ability to manipulate 'Sylvia' flowering time to fall within the optimum marketing period of September-January (Gerber *et al.*, 2001). During the 2001/2002 season 'Sylvia' protea accounted for >43% of exported cultivars (Table 2) (SAPPEX, 2002). That 'Sylvia' proteas comprise the largest *Protea* cultivar in production and the fact that it is particularly susceptible to leaf blackening directed the current research.

Leaf blackening in *Protea* cut flowers was extensively reviewed by Jones *et al.* (1995) and updated by van Doorn (2001). This literature review largely follows the format of these reviews with additional information where applicable.

Leaf blackening appearance

De Swardt (1979) defined leaf blackening as regions of dark brown to black discolouration appearing in various regions of the leaf. Four leaf blackening categories, based on the initial appearance of blackening, have been defined:

a. Leaf tip blackening;
b. Marginal blackening: originating at leaf edges and spreading inwards to the midrib;
c. Spot blackening: originating randomly on the leaf;
d. Midrib blackening: originating in the immediate vicinity of the midrib and spreading outwards to leaf margins (de Swardt, 1979).

The subsequent rapid blackening across the leaf irrespective of the site of initial leaf blackening would indicate that the same process is involved.

Leaf blackening may occur within several hours of harvest (de Swardt, 1979) although more commonly within two to five days postharvest (Newman *et al.*, 1990; McConchie *et al.*, 1991; Jones & Clayton-Greene, 1992). Leaf blackening has a negative effect on quality and is the most common cause for *Protea* cut flower consignment rejection and consequent decreased profitability (Perishable Products Export Control Board (PPECB), pers. comm.). Compounding this problem is the occurrence of preharvest leaf blackening (pers. observations).

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Preharvest leaf blackening

Preharvest leaf blackening of *Protea* leaves may be effected by water stress, mechanical damage and insect or fungal attack (Starke, 1979; Forsberg, 1993). Preharvest leaf blackening may be distinguished from that occurring postharvest by association of physical damage with blackened areas, usually at the center of the affected region or, in the case of exposure to excessive heat as a lighter brown colouration across the leaf.

Research on preharvest leaf blackening, unassociated with mechanical damage or fungal infection, has yet to be undertaken.

Postharvest leaf blackening

Susceptibility and severity of postharvest leaf blackening has been shown to vary between species (McConchie & Lang, 1993a), clones within a species and time of year (Paull & Dai, 1990). In Hawaii, leaf blackening of *P. neriifolia* is reportedly more severe in September (late summer) (Paull & Dai, 1990). Jones *et al.* (1995), citing unpublished data of van Doorn, stated that leaf blackening was frequently found in flowers exported from South Africa during February. However, South African producers and exporters and European markets report leaf blackening to be more problematic in *Protea* cut flowers during the months of September-November (spring) (Bergflora, pers. comm.).

Effect of preharvest conditions

De Swardt (1979) stated that shoots from plants grown under adverse conditions of water stress or those from aged plants were more susceptible to

postharvest leaf blackening. However, no data was presented to support this claim. Postharvest leaf blackening has also been negatively correlated with leaf concentrations of Mn, Zn and N in 13 *Protea* species (Akamine *et al.*, 1979). However, investigations into the effect of preharvest nutrition on concentrations of these minerals were not performed.

The time of day at which shoots were harvested was reported to correlate with postharvest leaf blackening which was reduced when shoots were harvested in the afternoon instead of morning (Paull & Dai, 1990). This was attributed to an increase in carbohydrate reserves that could be used in vase life extension (Jones *et al.*, 1995).

Physiological causes of leaf blackening

It has been hypothesised that leaf blackening in *Protea* is induced by stresses leading to breakdown of cellular membranes with the consequent substrate and enzyme interaction and resultant oxidation of polyphenol compounds by polyphenol oxidases (PPO) and peroxidases (POD) (de Swardt, 1979; Paull *et al.*, 1980; Whitehead & de Swardt, 1982; Ferreira, 1983). Whitehead & de Swardt (1982) reported on enzyme activities in leaves from plants maintained under a 12 h photoperiod in which no leaf blackening occurred. As discussed by Jones *et al.* (1995), the absence in these trials of the enzyme catalase to inhibit POD could possibly have resulted in phenolic compound oxidation by POD being incorrectly attributed to PPO activity. Research directed to the inhibition of both PPO and POD enzyme activity has been unsuccessful (du Toit, 1978; Newman *et al.*, 1990; Jones & Clayton-Greene, 1992).

Mulder (1983) reported a rapid degradation in cellular membranes in *P. neriifolia* selections susceptible to leaf blackening. It was suggested that the brown degradation products formed during chlorophyll breakdown could be responsible for leaf blackening.

McConchie and Lang (1993b) suggested that membrane integrity was maintained during leaf blackening as no degradation products were observed, antioxidant activity remained significantly unaltered and respiration rates remained relatively high. Although leaf blackening was significantly greater in shoots held in continuous darkness no significant difference was found in the *in vitro* activity of POD compared with shoots subjected to a 12 h light/12 h dark regime. Furthermore, McConchie *et al.* (1994) found PPO activity to be unrelated to appearance of blackening symptoms which, in the case of dark-held shoots, was approximately 30% greater than that of shoots exposed to light. The involvement of PPO in leaf blackening was reported by Dai & Paull (1997) who found a high *in vitro* PPO activity in leaf blackening susceptible *P. neriifolia* leaves which was absent in leaves of *Leucospermum* cv. Rachel [(*L. lineare* × *L. vestium*) × *L. glabrum*]. *Leucospermum* cv. Rachel exhibited no leaf blackening symptoms whilst the combination of 'Rachel' and *P. neriifolia* leaf extracts inhibited PPO activity in the latter (Dai & Paull, 1997). Two possible hypotheses were put forward, firstly that the *Leucospermum* extract contained an inhibitor of PPO activity or secondly, that the lower pH of the *Leucospermum* leaf extract (pH 4.4 compared to pH 5.2) prevented the processes involved in leaf blackening (Dai & Paull, 1997).

Suppression of leaf blackening by total immersion of shoots in an anti-oxidant diphenylamine (DPA) (1.5 $mg \cdot L^{-1}$) was caused either by inhibition of PPO

oxidation of phenols, or through inhibition of phenol oxidation by oxygen free-radicals (Jones & Clayton-Greene, 1992). At present a clear role for oxidative enzymes in *Protea* leaf blackening is lacking.

Water stress

The vase life of many cut flowers is limited by a reduced ability to take up water caused by vascular blockage possibly effected by cell metabolites, microbial growth or air embolism (Halevy & Mayak, 1981).

Several researchers have suggested that water loss from leaves, brought about by flowerhead transpiration, caused a water stress which damaged cell membranes and contributed to leaf blackening (de Swardt, 1979; Paull *et al.*, 1980; de Swardt & Pretorius, 1980; Ferreira, 1983; Paull & Dai, 1990). Du Plessis (1978) determined that leaf blackening of *P. neriifolia* was reduced when stem bases were recut and vase water replaced on a daily basis. Leached flavonoids were reported to be rapidly oxidised to form tannins (de Swardt *et al.*, 1987) and it was thought that uptake of these tannins could result in stem plugging and consequently water stress (Jacobs, 1981; de Swardt *et al.*, 1987). Use of lead acetate (du Plessis, 1978; de Swardt, 1979) and phenylmercury acetate (Masie, 1979) to precipitate these tannins led to a reduction in leaf blackening. However, as discussed by Jones *et al.* (1995), it is unclear from these findings as to whether the tannins effected stem occlusion or effected leaf blackening. Furthermore, it is unclear as to whether the precipitated compound effected either a reduction in vascular blockage or leaf blackening.

Inclusion of the anti-microbial compound 8-hydroxyquinoline sulfate (50-100 $mg \cdot L^{-1}$) in the vase solution delayed leaf blackening onset in *P. neriifolia* (Brink, 1987). In contrast, vase solutions incorporating anti-microbial compounds: sodium hypochlorite (Newman *et al.*, 1990; Bieleski *et al.*, 1992); silver thiosulfate and sodium sulfite (Newman *et al.,* 1990); trichloro-isocyanuric acid, hydroxyquinoline citrate and silver nitrate (van Doorn, 2001) at varying concentrations had no effect on *P. eximia* leaf blackening.

Enclosing *P. eximia* flowers in plastic bags, thereby reducing transpiration, or subjecting shoots to an overnight water stress (20°C, 60% RH) prior to vase life assessment had no effect on leaf blackening (Reid *et al.*, 1989). In contrast, application of anti-transpirants has been reported to reduce leaf blackening in *P. neriifolia* (Paull & Dai, 1990). Furthermore, blackening was enhanced in leaves in contact with free water inside bags (Reid *et al.*, 1989).

Although literature does not support the hypothesis that water stress causes leaf blackening of *Protea* cut flowers, a contributory role may currently not be excluded.

Carbohydrate stress

Starch and sucrose have been identified as the main non-structural metabolic carbohydrates in *Protea* (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie & Lang, 1993a,b) whilst glucose, fructose, sucrose and xylose form significant components in *Protea* nectar (Cowling & Mitchell, 1981; van Wyk & Nicholson, 1995). A further soluble carbohydrate found in *Protea* species is the sugar alcohol 1,5-anhydro-D-glucitol (polygalatol) (Boeyens *et al.*, 1983; Bieleski *et al.*, 1992;

McConchie & Lang, 1993a,b). Whilst starch and sucrose concentrations declined rapidly postharvest in the leaves of *P. neriifolia* and *P. eximia*, polygalatol levels did not change significantly even when sucrose and starch levels were low and leaf blackening present (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie & Lang, 1993a,b). The apparent unavailability of polygalatol for translocation and utilisation suggested that the major role for metabolically inert sugars was in osmotic buffering (Bieleski *et al.*, 1992).

Carbohydrate depletion in leaves, effected by requirements of the developing flowerhead and nectar production, was hypothesised to initiate leaf blackening (Ferreira, 1986; Paull & Dai, 1990; Dai, 1993) and was supported by the findings that flowerhead removal and girdling significantly reduced or delayed leaf blackening (Paull *et al.,* 1980; Brink & de Swardt, 1986; Brink, 1987; Reid *et al.*, 1989; Paull & Dai, 1990; Dai, 1993; Tranter, 1989; Dai & Paull, 1995). In contrast, Bieleski *et al.* (1992) reported that leaf blackening in *P. eximia* did not differ significantly between deheaded or intact shoots. This apparent contradiction may have been due either to the delay in experimental set-up, during which time carbohydrate may have been rapidly depleted, or that shoots were held in darkness. Shoots harvested with less developed inflorescences had a higher incidence of leaf blackening than that of shoots with more developed inflorescences (Paull & Dai, 1990). This was partially explained by the finding that respiration rates were significantly higher in more immature inflorescences (Ferreira, 1986). Leaf blackening was correlated with increasing temperature (Jacobs & Minaar, 1977a), which may be attributed to increased respiratory demands of the flowerhead. Leaf blackening was significantly suppressed in *P. neriifolia* cut flowers placed in a low

oxygen, high carbon dioxide (1% O_2; 5% CO_2) atmosphere for 14 d (5 $\mu mol \cdot m^{-2} \cdot s^{-2}$; 25°C, 90% RH) (Jones & Clayton-Greene, 1992). However, when shoots were removed to an ambient atmosphere leaf blackening progressed rapidly indicating that although the low oxygen atmosphere inhibited the final oxidation of phenols it did not inhibit the processes that lead to this stage (Jones & Clayton-Greene, 1992).

Several *Protea* species produce significant nectar volumes (Mostert *et al.*, 1980; Cowling & Mitchell, 1981; Wiens *et al.*, 1983). Subsequent to a 24 h application of [^{14}C] sucrose to *P. neriifolia* stems more than 50% of the radioactivity was found in the nectar (Dai, 1993) which led to the hypothesis that photosynthate translocation from the leaves to developing flowerhead initiated leaf blackening (Newman e*t al.*, 1990).

The onset of postharvest leaf blackening has been correlated with reduced leaf carbohydrate content (McConchie *et al.*, 1991, 1994; Jones & Clayton-Greene, 1992; Bieleski *et al.*, 1992; McConchie & Lang, 1993b). Under lighted conditions carbon assimilates and reserves in *P. neriifolia* shoots were converted to transport carbohydrates during flowerhead development (McConchie *et al.*, 1991). McConchie & Lang (1993b) reported that more than 70% of pre-harvest starch concentration in leaves was depleted within 24 h of harvest. Furthermore, removal of the flowerhead subsequent to 24 h dark storage did not significantly reduce leaf blackening (McConchie & Lang, 1993b).

Several papers have investigated the role of photosynthesis and light in postharvest leaf blackening and demonstrated a delay in leaf blackening in shoots

held under sufficient light to allow carbon assimilation (Newman *et al.*, 1990; Paull & Dai, 1990; McConchie *et al.*, 1991; Jones & Clayton-Greene, 1992; Bieleski *et al.*, 1992). A significant reduction in leaf blackening of *P. eximia* cut flowers was reported in shoots subjected to photosynthetically active radiation (PAR) (15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Newman *et al.*, 1990). Exposure to unspecified light levels reduced leaf blackening of *P. neriifolia*, *P. compacta* and *P. eximia* (Jacobs & Minaar, 1977b; La Rue & La Rue, 1986). Exposure of *P. neriifolia* to light intensities ≥ 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ significantly inhibited leaf blackening (Jones & Clayton-Greene, 1992). Inhibition of the photosynthetic electron transport chain of photosystem II by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), resulted in a significant increase in leaf blackening which indicated the importance of photosynthesis in leaf blackening inhibition (Jones & Clayton-Greene, 1992).

Carbohydrate supplementation is a recognised practice in storage and vase life extension of many cut flower crops (Halevy & Mayak, 1974, 1979, 1981; Goszczyñska & Rudnicki, 1988; Nowak *et al.*, 1990). Use of exogenous sugars in *Protea* cut flowers has only been partially successful. Sucrose holding solutions (≤ 2 $g \cdot L^{-1}$) have been reported to effectively reduce postharvest leaf blackening of *P. compacta* (Ireland *et al.*, 1967; Haasbroek *et al.*, 1973), *P. eximia* (Ireland *et al.*, 1967; Bieleski *et al.*, 1992), *P. cynaroides* and *P. magnifica* (Ireland *et al.*, 1967) and *P. neriifolia* (Mulder, 1977; Brink & de Swardt, 1986; Brink, 1987; Paull & Dai, 1990; McConchie *et al.*, 1991). Holding solutions with sucrose at higher concentrations exacerbated *P. neriifolia* leaf blackening (Brink, 1987; Jones, 1991a). In contrast a 30 $g \cdot L^{-1}$ sucrose holding solution significantly suppressed leaf

blackening of *P. eximia* (Akamine *et al.*, 1979). Sucrose pulsing solutions (200 $g \cdot L^{-1}$, 24 h, 1°C) significantly reduced leaf blackening of *P. cynaroides* during long-term dry storage (1°C) (Jones, 1991a). A similar benefit was found in *P. neriifolia* pulsed with sucrose (200 $g \cdot L^{-1}$, 24 h, 25°C) prior to seven days of dark, wet storage at 25°C (McConchie & Lang, 1993b).

Supply of exogenous sugars retards senescent processes in many cut flower crops by maintaining cellular membrane integrity and mitochondrial function, and by delaying degradation of protein and ribonucleic acid (Halevy & Mayak, 1979; Nowak *et al.*, 1990). Leaf blackening induced by gamma irradiation was inhibited by a 3.5% sucrose solution, indicating a possible reduction in cellular membrane damage caused by irradiation (Haasbroek *et al.*, 1973). *Leucadendron* cv. Silvan Red (*L. laureolum* × *L. salignum*) leaves on shoots subjected to a sucrose pulse treatment (200 $g \cdot L^{-1}$, 24 h, 1°C) were protected from desiccation during 42 d storage at 1°C (Jones, 1991b).

The effect of carbohydrate supplementation on both transport and subsequent vase life and a direct comparison between species and cultivars is lacking in literature.

Ethylene sensitivity

Ethylene has a detrimental effect on the postharvest vase life of many cut flower products and plays an important role in processes associated with flower senescence (Halevy & Mayak, 1979, 1981). Pre-treatment of *P. eximia* shoots with 4 mM silver thiosulfate, an inhibitor of ethylene action, did not suppress leaf

blackening (Newman *et al.*, 1990; Bieleski *et al.*, 1992). Ethylene evolution was not detected (<0.01 $\mu L \cdot L^{-1}$) in *P. neriifolia* leaves during postharvest assessment (McConchie & Lang, 1993b). However, van Doorn (2001) suggested that the presence of ethylene generating fruit, e.g. apples, through storage and transport might increase leaf blackening.

Biochemistry of leaf blackening

Several phenolic compounds are found in the Proteaceae (van Rheede van Oudtshoorn, 1963; Elsworth & Martin, 1971; Perold *et al.*, 1973a,b; Perold *et al.*, 1979; Perold & Carlton, 1989; Perold, 1993). In leaves of *Protea* species susceptible to leaf blackening unstable *O*-glycoside esters, formed from β-D-sugars such as glucose and allose, and aglycones, typically di- and trihydroxybenzene derivatives have been identified (Perold *et al.*, 1973a,b; Perold *et al.*, 1979, Perold & Carlton, 1989, Perold, 1993). In contrast, the non-blackening Proteaceae members contain stable *C*-glycoside esters (Elsworth & Martin, 1971; Perold, 1993). Hydrolysis of phenolic glucoside esters by glucosidase enzymes results in the formation of a free sugar and a reactive phenolic moiety (Dey & Dixon, 1985). Perold (1993) hypothesised that the demand for carbohydrates for the developing flowerhead may stimulate or cause the cleavage of these esters allowing the sugar(s) to be translocated to the flowerhead and releasing a reactive phenolic moiety which can undergo non-enzymatic oxidation resulting in leaf blackening. Glycosidase enzymes, e.g. β-D-glucosidase, occur in plant vacuoles (Marty *et al.*, 1980) as do phenolic glucosides (Lagrimi, 1992) which suggests that membrane degradation is

not a pre-requisite for enzyme-substrate interaction and concurs with McConchie *et al.* (1994) who reported that leaf blackening progressed without cellular membrane disintegration.

Activity of β-D-glycosidase in attached leaves of 'Pink Ice' proteas increased significantly prior to the appearance of leaf blackening (Jones & Cass, 1996). Leaves on shoots held under lighted conditions did not blacken and β-D-glycosidase activity remained constant (Jones & Cass, 1996). *In vitro* inhibition of β-D-glycosidase was achieved by solutions containing Cu^{2+} and Zn^{2+} but when these were supplied through stems they were ineffective in preventing and, at certain concentrations actually enhanced, leaf blackening of 'Pink Ice' proteas (Jones & Cass, 1996). Immersion of entire shoots in either 20 or 50% ethanol solutions significantly delayed the onset of leaf blackening, however when supplied either as a pulse or holding solution these were ineffective (Jones & Cass, 1996). Ethanol vapour significantly reduced leaf blackening of 'Pink Ice' proteas held for 19 d (20°C) inside plastic bags although no data was presented on leaf blackening subsequent to bag removal (Crick & McConchie, 1999).

Conclusion

It would appear that carbohydrate depletion in the leaves, effected by flowerhead demands, is a major factor in leaf blackening of *Protea* cut flowers. Although several reports outline treatments to suppress leaf blackening these have yet to be employed in trials covering both transport and subsequent vase life

assessment. At present, no standard commercial practice exists to prevent or suppress leaf blackening of *Protea* cut flowers.

Literature cited

Akamine, E.K., Goo, T. & Suehisa, R., 1979. Relationship between leaf darkening and chemical composition of leaves of Protea. Flor. Rev. 163, 62-63, 107-108.

Bieleski, R.L., Ripperda, J., Newman, J.P. & Reid, M.S., 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117, 124-127.

Boeyens, J.C.A, Marais, J.L.C. & Perold, G.W., 1983. The occurrence, conformation and crystal structure of 1,5-anhydro-D-glucitol in *Protea* spp.. Phytochem. 22, 1959-1960.

Brink, J.A., 1987. The influence of the flower-head on leaf browning of cut Protea *neriifolia*. Protea News 5, 11-12.

Brink, J.A. & de Swardt, G.H., 1986. The effect of sucrose in vase solution on leaf browning of *Protea neriifolia* R. Br.. Acta Hort. 185, 111-119.

Cowling, R.M. & Mitchell, D.T., 1981. Sugar composition, total nitrogen and accumulation of C_{14} assimilates in floral nectaries of *Protea* species. J. S. Afr. Bot. 47, 743-750.

Crick, S.G. & McConchie, R., 1999. Ethanol vapour reduces leaf blackening in cut flower *Protea* 'Pink Ice' stems. Postharvest Biol. Technol. 17, 227-231.

Dai, J-W., 1993. Postharvest leaf blackening in *Protea neriifolia* R. Br.. Ph. D. Diss., Univ. of Hawaii at Manoa, Honolulu.

Dai, J-W. & Paull, R.E., 1995. Source-Sink relationship and *Protea* postharvest leaf blackening. J. Amer. Soc. Hort. Sci. 120, 475-480.

Dai, J-W. & Paull, R.E., 1997. Comparison of leaf susceptibility to enzymatic blackening in *Protea neriifolia* R. Br. and *Leucospermum* 'Rachel'. Postharvest Biol. Technol. 11, 101-106.

De Swardt, G.H., 1979. Blackening of *Protea* leaves. SAPPEX Res. Paper 13.

De Swardt, G.H. & Pretorius, J., 1980. Browning of foliage leaves in proteas. Farming in South Africa: Flowers and Ornamentals Publ. B.13

De Swardt, G.H., Pretorius, J. & Burger, L., 1987. The browning of foliage leaves in Proteas – a review. Protea News 6, 4-9.

Dey, P.M. & Dixon, R.A., 1985. Glycosides. In: P.M. Dey & R.A. Dixon (eds), Biochemistry of storage carbohydrates in green plants. Academic Press, New York. pp 131-148.

Du Plessis, D.G.C., 1978. Leuko-antosianienbinders as beheermaatreël vir die voorkoming van bruinwording in Protealoofblare. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.

Du Toit, S., 1978. Die moontlike bekamping van loofblaarverbruining by *Protea neriifolia* met difenielamiensulfoonsuur. M.Sc. Thesis, Randse Afrikaanse Univ., Johannesburg, South Africa.

Elsworth, J.F. & Martin, K.R., 1971. Flavonoids of the Proteaceae. Part I. A chemical contribution to studies on the evolutionary relationships in the South African Proteoideae. J. S. Afr. Bot. 37, 199-212.

Ferreira, D.I., 1983. Prevention of browning of leaves of *Protea neriifolia* R. Br.. Acta Hort. 138, 273-276.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescences. Acta Hort. 185, 121-129.

Forsberg, L.I., 1993. Protea diseases and their control. Queensland Dept Primary Industries, Brisbane, Queensland.

Gerber, A.I., Theron, K.I. & Jacobs, G., 2001. Manipulation of flowering time by pruning of *Protea* cv. Sylvia. HortScience 36, 909-912.

Goszczyñska, D.M. & Rudnicki, R.M., 1988. Storage of cut flowers. Hort. Rev. 10, 35-62.

Haasbroek, F.J., Rousseau, G.G. & de Villiers, J.F., 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5, 33-42.

Halevy, A.H. 7 Mayak, S., 1974. Transport and conditioning of cut flowers. Acta Hort. 43, 291-306.

Halevy, A.H. & Mayak, S., 1979. Senescence and postharvest physiology of cut flowers. Part 1. Hort. Rev. 1, 204-236.

Halevy, A.H. & Mayak, S., 1981. Senescence and postharvest physiology of cut flowers. Part 2. Hort. Rev. 3, 59-143.

Ireland, J.P., Meynhardt, J.T. & Strauss, J.M., 1967. When proteas become sailors: treatment before shipping. Farming in South Africa 43, 33-35.

Jacobs, G., 1981. Post-harvest handling of proteas. In: P. Matthews (ed.), Growing and marketing of proteas. ProteaFlora Enterprises, Melbourne, Australia. pp 40-52.

Jacobs, G. & Minaar, H.R., 1977a. Effect of temperature on the blackening of *Protea* leaves. SAPPEX Newsletter 17, 20-24.

Jacobs, G. & Minaar, H.R., 1977b. Effect of light on the blackening of *Protea* leaves. SAPPEX Newsletter 18, 18-20.

Johnson, L.A.S. & Briggs, B.G., 1975. On the Proteaceae - the evolution and classification of a southern family. Bot. J. Linn. Soc. 70, 83-182.

Jones, R.B., 1991a. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. Proc. Int. Protea Assoc. 6th Biennial Conf., Perth, Western Australia. pp 313-322.

Jones, R.B., 1991b. A pre-storage sucrose pulse protects *Leucadendron* var. 'Silvan Red' during long term dry storage at 1°C. Acta Hort. 298, 247-253.

Jones, R.B. & Clayton-Greene, K.A., 1992. The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia* R. Br. leaves. Scientia Hort. 50, 137-145.

Jones, R.B. & Cass, A., 1996. The possible involvement of a β-D-glucosidase in postharvest leaf blackening in cut *Protea* flowers. J. Int. Protea Assoc. 31, 36-44.

Jones, R.B., McConchie, R., van Doorn, W.G. & Reid, M.S., 1995. Leaf blackening in cut *Protea* flowers. Hort. Rev. 17, 173-201.

La Rue, B.H. & La Rue, R.L., 1986. Postharvest information from a *Protea* grower, shipper and importer's point of view. Acta Hort. 185, 131-136.

Lagrimi, L.M., 1992. Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. Plant Physiol. 96, 577-583.

Marty, F., Branton, D. & Leigh, R.A., 1980. Plant vacuoles. In: N.E. Tolbert (ed.) The biochemistry of plants. Vol. 1. Academic Press, New York. pp 625-658.

Masie, W.E., 1979. Fenielmerkuri-asetaat as voorkomingsmiddel vir loofblaarverbruining by *Protea neriifolia* R.Br.. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.

McConchie, R., Lang, N.S. & Gross, K.C., 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116, 1019-1024.

McConchie, R. & Lang, N.S., 1993a. Postharvest leaf blackening and pre-harvest carbohydrate status in three *Protea* species. HortScience 28, 313-316.

McConchie, R. & Lang, N.S., 1993b. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118, 355-361.

McConchie, R., Lang, N.S., Lax, A.R. & Lang, G.A., 1994. Re-examining polyphenol oxidase, peroxidase, and leaf blackening in *Protea*. J. Amer. Soc. Hort. Sci. 119, 1248-1254.

Mostert, D.P., Siegfried, W.F. & Louw, G.N., 1980. *Protea* nectar and satellite fauna in relation to the food requirements and pollinating role of the Cape sugarbird. S. Afr. J. Sci. 76, 409-412.

Mulder, P.W.A., 1977. Primêre meganismes betrokke by die bruinwording van loofblare in *Protea neriifolia*. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.

Mulder, P.W.A, 1983. Die rol van flavonoïde op die sensitiwiteit vir loofblaarverbruining by verskillende variante van *Protea neriifolia* R.Br.. Ph.D. Diss., Randse Afrikaans Univ., Johannesburg, South Africa.

Newman, J.P., van Doorn, W. & Reid, M.S., 1990. Carbohydrate stress causes leaf blackening in Proteas. Acta Hort. 264, 103-108.

Nowak, J., Rudnicki, R.M. & Duncan, A.A., 1990. Postharvest handling and storage of cut flowers, florist greens, and potted plants. Timber Press, Portland, Oregon.

Paull, R.E., Goo, T., Criley, R.A. & Parvin, P.E., 1980. Leaf blackening in cut *Protea eximia*: importance of water relations. Acta Hort. 113, 159-166.

Paull, R.E. & Dai, J-W., 1990. *Protea* postharvest black leaf a problem in search of a solution. Acta Hort. 264, 93-101.

Perold, G.W., 1993. Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. S. Afr. J. Sci. 89, 90-93.

Perold, G.W., Beylis, P. & Howard, A.S., 1973a. Metabolites of Proteaceae. Part VII. Lacticolorin, a phenolic glucoside ester and other metabolites of *P. lacticolor* Salisb.. J. Chem. Soc. Perkin Trans I 1973, 638-642.

Perold, G.W., Beylis, P. & Howard, A.S., 1973b. Metabolites of Proteaceae. Part VIII. The occurrence of (+)-D-allose in nature: rubropilosin and pilorubrosin from *Protea rubropilosa* Beard. J. Chem. Soc. Perkin Trans I 1973, 643-649.

Perold, G.W., Rosenburg, M.E.K., Howard, A.S. & Huddle, P.A., 1979. Metabolites of Proteaceae. Part 9. Eximin (6-O-benzoylarbutin) and the synthesis of aryl glycoside esters. J. Chem. Soc. Perkin Trans I 1979, 239-243.

Perold, G.W. & Carlton, L., 1989. Neriifolin, an ester glucoside of benzene-1,2,4-triol. J. Chem. Soc. Perkin Trans I 1989, 1215-1217.

Reid, M.S., van Doorn, W.G. & Newman, J.P., 1989. Leaf blackening in proteas. Acta Hort. 261, 81-84.

SAPPEX, 1995. Export season July 1994-June 1995: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

SAPPEX, 1999. Export season July 1998-June 1999: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

SAPPEX, 2000. Export season July 1999-June 2000: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

SAPPEX, 2001. Export season July 2000-June 2001: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

SAPPEX, 2002. Export season July 2001-June 2002: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

Starke, L.C., 1979. Common *Protea* pests. Farming in South Africa: Flowers and Ornamental Shrubs Publ. B.11.

Tranter, D., 1989. Postharvest blackleaf in the *Protea* cultivar 'Pink Ice'. J. Int. Protea Assoc. 17, 13-20.

Van Doorn, W.G., 2001. Leaf blackening in *Protea* flowers: recent developments. Acta Hort. 545, 197-204.

Van Rheede van Oudtshoorn, M.C.B., 1963. Distribution of phenolic compounds in some South African Proteaceae: a contribution to the chemotaxonomy of the family. Planta Med. 4, 399-406.

Van Wyk, B-E & Nicholson, S.W., 1995. Xylose is a major nectar sugar in *Protea* and *Faurea*. J. S. Afr. Sci., 91, 151-152.

Whitehead, C.S. & de Swardt, G.H., 1982. Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of *Protea neriifolia*. S. Afr. J. Bot. 1, 127-130.

Wiens, D., Rourke, J.P., Casper, B.B., Rickart, E.A., LaPine, T.R., Peterson, C.J. & Channing, A., 1983. Nonflying mammal pollination of southern African proteas: a noncoevolved system. Ann. Missouri Bot. Gard. 70, 1-31.

Tables

Table 1.

SAPPEX export figures for *Protea* cv. Sylvia stems.

Export season[a]	Number of stems exported
1994/1995	Unspecified
1998/1999	107 737
1999/2000	93 369
2000/2001	328 820
2001/2002	759 569

[a] season runs from July to June of the following year

(SAPPEX, 1995, 1999-2002).

Table 2.

SAPPEX *Protea* cultivar export figures. Export season July 2001-June 2002.

Cultivar	Number of stems exported
'Brenda'	9 840
'Cardinal'	289 752
'Jupiter'	800
'Liebencherry'	15 508
'Niobe'	2 450
'Pink Duke'	820
'Pink Ice'	331 234
'Pinita'	880
'Sheena'	1 168
'Sheila'	76 175
'Susara'	261 428
'Sylvia'	759 569

(SAPPEX, 2002).

2. PAPER I - Effect of supplemental postharvest photoperiodic light, storage temperature, ethylene and girdling on vase life of 'Sylvia' protea cut flowers

Effect of post-harvest supplemental photoperiodic light, storage temperature, ethylene and girdling on vase life of 'Sylvia' protea cut flowers

Leaf blackening is thought to involve carbohydrate depletion developing in the leaves due to demands of the flowerhead which continues to develop after harvest (Reid *et al.*, 1989; Tranter, 1989; Newman *et al.*, 1990; McConchie *et al.*, 1991, 1994; Jones & Clayton-Greene, 1992; Bieleski *et al.*, 1992; McConchie & Lang, 1993; Dai & Paull, 1995).

In shoots held under sufficient light to allow carbohydrate assimilation, leaf blackening has been significantly delayed (Newman *et al.*, 1990; Paull & Dai, 1990; McConchie *et al.*, 1991; Jones & Clayton-Greene, 1992; Bieleski *et al.*, 1992). Under lighted conditions carbon assimilates and reserves in *Protea neriifolia* were converted to transport carbohydrates during flowerhead development (McConchie *et al.*, 1991). Removal of the flowerhead and girdling directly below the flowerhead reduced or delayed leaf blackening in *P. eximia* (Paull *et al.*, 1980; Reid *et al.*, 1989) and *P. neriifolia* (Brink & de Swardt, 1986; Brink, 1987; Paull & Dai, 1990; Dai, 1993; Tranter, 1989; Dai & Paull, 1995). Starch concentration within leaves rapidly decreased post harvest, e.g. more than 70% of preharvest starch concentration was depleted within 24 h of harvest in *P. neriifolia* (McConchie & Lang, 1993). Leaf blackening was significantly reduced in *P. eximia* cut flowers subjected to

photosynthetically active radiation (PAR) (15 $\mu mol \cdot m^{-2} \cdot s^{-1}$), whilst exposure to higher, unspecified light intensities either reduced or completely suppressed leaf blackening in *P. repens*, *P. cynaroides*, *P. neriifolia* and *P. punctata* (Newman *et al.*, 1990). Exposure to unspecified light levels was reported to reduce leaf blackening of *P. neriifolia*, *P. compacta* and *P. eximia* (Jacobs & Minaar, 1977b). Inhibition of the photosynthetic electron transport chain of photosystem II by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), caused a significant increase in leaf blackening which indicated the importance of photosynthesis in leaf blackening inhibition (Jones & Clayton-Greene, 1992).

Low temperatures throughout the distribution chain are recommended for many cut flower crops (Lutz & Hardenburg, 1968, Halevy & Mayak, 1981; Mayak, 1985; Hardenburg *et al.*, 1986; Goszczyñska & Rudnicki, 1988; Nowak *et al.*, 1990; Reid, 1992). Storage and vase life can be beneficially extended by low temperature due to suppression of physiological processes associated with senescence, respiration rate (Reid & Kofranek, 1980) and water loss (Faragher *et al.*, 1984). However, the varied response of cut flower crops to low temperature conditions indicates a need to determine optimal temperatures for individual species and cultivars in order to prevent possible chilling injury and maximise storage potential (Levitt, 1972; Goszczyñska & Rudnicki, 1988).

Leaf blackening was positively correlated with increasing temperature and was attributed to increased respiratory demands of the flowerhead (Jacobs & Minaar, 1977a). Vase life of several *Protea* species has been extended subsequent to storage at

2°C (Ireland *et al.*, 1967; Haasbroek *et al.*, 1973; Meynhardt, 1976; Paull *et al.*, 1980; Jacobs, 1981). Meynhardt (1976) reported a satisfactory vase life for several Proteaceae subsequent to six weeks storage at 2°C. In contrast, Jacobs (1981) reported that vase life in many of the same cut flowers was unsatisfactory after three weeks storage at 2°C. A lower storage temperature (1°C) was found beneficial in maintaining satisfactory vase life in *P. cynaroides* (Jones, 1991) whilst for *P. neriifolia* results were variable (Jones & Faragher, 1991; Shelton *et al.*, 1996). Despite this most cut Proteaceae products from South Africa are currently exported at a minimum temperature of 4°C and frequently temperatures exceed this minimum.

Ethylene has a detrimental effect on the postharvest vase life of many cut flower crops and plays an important role in processes associated with flower senescence (Halevy & Mayak, 1979, 1981). Pre-treatment of *P. eximia* shoots with 4 mM silver thiosulfate did not suppress leaf blackening (Newman *et al.*, 1990; Bieleski *et al.*, 1992). Ethylene evolution was not detected (<0.1 $\mu L \cdot L^{-1}$) in *P. neriifolia* leaves during postharvest assessment (McConchie & Lang, 1993). However, van Doorn (2001) suggested that the presence of fruit, e.g. apples, which are known to generate ethylene during ripening, through postharvest storage and transport increased *Protea* leaf blackening. Exposure of 'Sylvia' proteas to ethylene was done to determine the role of ethylene in *Protea* leaf blackening.

The significant increase in cost and the unavailability of airfreight space for cut flowers directed investigations into shipping 'Sylvia' proteas by sea to European markets, a process which requires 21 d from the time of product delivery to the local

exporter to arrival on Europe markets. Export of 'Sylvia' proteas accounted for more than 43% of *Protea* cultivars exported in the 2001/2002 season (SAPPEX, 2002). The increased plantings of 'Sylvia' proteas may be attributed to the fact that it is one of the few *Protea* cultivars whose flowering time can be manipulated to fall within the desired marketing time from September to January (Gerber *et al.*, 2001).

Supplemental light, low temperatures, rapid cooling and girdling were evaluated as potential techniques to extend longevity and post-storage vase life of 'Sylvia' protea cut flowers.

Materials and Methods

PLANT MATERIAL. Flower-bearing shoots of 'Sylvia' proteas were harvested from a commercial plantation, Protea Heights, located in the Stellenbosch district, (33°55'S; 18°50'E), South Africa. The climate is Mediterranean with hot, dry summers and cold, wet winters. Shoots were harvested into water and brought to the laboratory within 1 hour of harvest. On arrival shoots were cut to 50 cm in length and the bottom 20 cm stripped of leaves.

SUPPLEMENTAL POSTHARVEST PHOTOPERIODIC LIGHT. To determine the effect of photoperiodic light on vase life, shoots were subjected to a light regime (12 h light/12 h dark) or darkness in a controlled temperature room (18±1°C) for 10 d. High pressure sodium (HPS) lamps (400W, SON-T; Osram, Munich, Germany) were used to deliver photoperiodic light at an intensity of 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at inflorescence

level, as determined by a Decagon light meter (AccuPAR ver. 4.1; Decagon Devices, Pullman, WA). Shoots were also subjected to one of the following treatments:

i) dark storage (72 h, 4.5°C)

ii) dark storage (72 h, 4.5°C); light (12 h, 18°C)

iii) light (12 h, 18°C); dark storage (72 h, 4.5°C)

iv) light (12 h, 18°C); dark storage (72 h, 4.5°C); light (12 h, 18°C)

Prior to dark storage treatment (72 h, 4.5°C), shoots were cooled for 8 h in cold rooms maintained at 4.5°C, then packed into SAPPEX S14 fibreboard cartons with lids.

After dark storage, shoots were recut to 50 cm and placed in individual vases containing tap water. Vase life was assessed in a controlled temperature room (18±1°C) for 10 d. Number of leaves with at least 10% blackened leaf area was determined at 2 d intervals. Ten single shoot replicates per treatment were used.

STORAGE TEMPERATURE. Shoots were placed in tap water and cooled for 8 h in cold rooms maintained at 0, 4.5, 7 and 10°C, then packed into SAPPEX S14 fibreboard cartons with lids. Cartons were then stored at the temperatures described for 3 d. After 3 d storage, stems were recut to 45 cm and placed in individual vases containing tap water and vase life assessed as previously described for 7 d. Six replicates of ten shoots per treatment were used.

RESPIRATION. Shoots harvested and cooled as described above were placed in containers attached to a continuous flow of humidified air maintained at a rate of 300

$mL \cdot min^{-1}$ using flow boards (Morris, 1969) and needle valves for 3 d. Respiration was measured 12, 24, 48 and 72 h after being placed in cold rooms described using an infrared gas analyser (IRGA) (model S-151; Qubit Systems, Kingston, Ontario). Four replications of five shoots per treatment were used.

A second experiment was executed to measure the relative contribution to total respiration by the flowerhead and leaves. Shoots that had been either completely stripped of leaves or had the flowerhead removed were used with intact shoots as the control treatment. Shoots described were placed in containers attached to the flowboards at 0 and 4.5°C for 96 h. Respiration was determined 24, 48, 72 and 96 h after being placed inside cold rooms using an IRGA (model S-151, Qubit Systems). Four replications of five shoots per treatment were used.

ETHYLENE. Shoots were placed in buckets containing tap water and cooled inside rooms maintained at 1°C for 6 h. Shoots were then placed in containers attached to a continuous flow of humidified air with 50 $\mu L \cdot L^{-1}$ ethylene or humidified air without ethylene at a rate of 300 $mL \cdot min^{-1}$ using flow boards (Morris, 1969) and needle valves. After 3 d storage (1°C) shoots were removed from containers and recut to 45 cm prior to placement in individual vases containing tap water. Vase life was assessed in a temperature controlled room (18±1°C) for 10 d. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Four replications of six shoots per treatment were used.

CARBOHYDRATE ANALYSIS. Shoots were stripped of all but subtending flush leaves then cooled and stored inside cold rooms maintained at 0, 4.5, 7 and 10°C as previously described. At harvest and after 3 d storage at the different temperatures leaves were lyophilised and dry mass determined before being milled to a fine powder. A 0.5 g sample of the dried tissue described was taken for carbohydrate analysis. Samples were extracted for 15 h in 1% acetic acid by shaking before being centrifuged (10,000 g_n, 12 min, 4°C). Supernatant was filtered and made up to 100 mL with 1% acetic acid. Thereafter the pellet was resuspended in an acetate buffer (pH 4.8) and gelatinised in a boiling steam bath for 2 h. The suspension was cooled to 60°C and the starch fraction hydrolysed to glucose with amyloglucosidase (EC 3.2.1.3) (Fluka Chemie, Buchs, Switzerland). Hydrolysis was performed in an incubator maintained at 55°C for 18 h. Analysis of reducing sugars and starch was done on a Sanplus Segmented Flow Analysis System (method numbers 551-965w/r issue 070798/MH and 356-001w/r issue 012998/MH97203066; Skalar, De Breda, The Netherlands). Six single shoot replicates per treatment were used.

QUANTIFICATION OF LEAF PHENOLIC COMPOUNDS. Shoot growth in 'Sylvia' proteas occurs in flushes named according to the season in which they are formed. In order to investigate phenolic compound concentration in different flushes a distinction was made between the flush on which the flowerhead was borne (subtending flush) and flushes formed earlier in shoot development (older flushes) (Fig.1).

Shoots that were harvested, cooled and stored inside cold rooms maintained at 0, 4.5, 7 and 10°C as previously described were used. Determination of phenolic concentration was based on the method described by Wand (1995). Phenolic

concentration of leaves from subtending and older flushes was measured at harvest and after 3 d storage at the different temperatures. Leaves were removed at point of inception, lyophilised and the dry mass determined before being milled to a fine powder. A 0.25 g sample of the dried tissue described was used for quantification of phenolic compounds. Samples were extracted for 15 h in 5 mL of methanol:water:HCl (79:20:1, v:v:v) and centrifuged (10,000 g_n, 12 min, 4°C). The pellet was resuspended in 5 mL extraction medium and centrifuged again (10,000 g_n, 12 min, 4°C). Absorbance of combined extracts was measured with a scanning spectrophotometer (model DU® series 64; Beckman Instruments, Irvine, CA) at 280, 300 and 360 nm. Absorbance values were expressed on a dry mass basis. Six single shoot replicates per treatment were used.

REMOVAL OF UPPERMOST LEAVES AND GIRDLING. Shoots had either the ±6 uppermost leaves removed or were girdled with a budding knife immediately beneath the flowerhead. Intact shoots served as control treatment. Shoots were placed in tap water and cooled for 8 h in cold rooms maintained at either 0 or 4.5°C. Cooled shoots were packed into SAPPEX S14 cartons with lids and stored at either 0 or 4.5°C for 3 d. After 3, 7, 14 and 21 d storage stems were recut to 45 cm and placed in individual vases containing tap water. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light for 10 d. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Six replicates of 10 shoots per treatment were used.

EFFECT OF COOLING METHOD ON POST-STORAGE VASE LIFE. Shoots were packed into SAPPEX S14 fibreboard cartons and either vacuum cooled to 1°C or subjected to forced air cooling at 1°C for either 30 or 60 minutes prior to 3 d storage (1°C, 95% RH). Upon removal from storage stems were recut to 45 cm and placed in individual vases containing tap water. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light for 8 d. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Four replications of ten shoots per treatment were used.

STATISTICAL ANALYSIS. Standard analysis of variance was performed on the data using the General Linear Model procedure generated by the SAS® program (Statistical Analysis Systems Institute, 1996). Students t-LSD were calculated at a 5% significance level to compare treatment means.

Results

SUPPLEMENTAL POSTHARVEST PHOTOPERIODIC LIGHT. Leaf blackening was significantly higher in shoots held in the dark at 18°C for 10 d than shoots held under an alternating photoperiodic light regime (12 h light/12 h dark) (Table 1). Leaf blackening was significantly higher in shoots held under 12 h photoperiodic light (18°C) prior to 72 h dark storage (4.5°C) compared to shoots placed immediately into storage at 4.5°C (Fig. 2).

STORAGE TEMPERATURE. Leaf blackening increased significantly with storage temperature (Fig. 3). Shoots stored at 0°C had a significantly lower number of blackened leaves and delayed appearance of leaf blackening. No symptoms of chilling injury were observed.

RESPIRATION. Respiration rates decreased significantly with lower storage temperature (Fig. 4). A significant decrease in respiration rate over time was found in those shoots stored above 0°C. It was determined that the flowerhead was responsible for the most significant portion of respiration (Fig. 5).

ETHYLENE. No significant difference in vase life between flowers treated with ethylene or air was found (data not presented).

CARBOHYDRATE ANALYSIS. Starch and reducing sugar content decreased significantly with increasing temperature (Table 2). There was no significant difference in starch and reducing sugar content of leaves stored at 0°C for 3 d and that at harvest.

QUANTIFICATION OF LEAF PHENOLIC COMPOUNDS. Phenolic concentration was significantly greater after storage although no significant difference was found between storage temperatures (Table 3). Phenolic concentration was higher in leaves from the subtending flush than older flushes.

REMOVAL OF UPPERMOST LEAVES AND GIRDLING. Removal of the uppermost ±6 subtending flush leaves was found to significantly delay onset of leaf blackening in shoots stored for 3 d at 0 and 4.5°C (Table 4). However, no significant difference in number of blackened leaves was found for longer storage periods. Girdling significantly reduced the number of blackened leaves and onset of leaf blackening for each storage

period (Fig. 6). Leaf blackening increased significantly with length of storage. Leaf blackening was significantly lower in shoots stored at 0°C.

Vase life of girdled shoots held at 0°C for 3 d was maintained for 10 d, whilst that of shoots stored at 4.5°C was limited by involucral bract browning and collapse to 7 d. Onset of leaf blackening was significantly delayed by 3 d for intact shoots held at 0°C. The effects of storage temperature and girdling treatments on 'Sylvia' protea cut flower appearance are shown in Figs 7 & 8. Excessive involucral bract browning and collapse of girdled shoots held at 4.5°C for 14 d limited vase life to 4 d. Leaves of intact shoots stored at 4.5°C for 21 d were completely blackened upon removal from storage. Blackening of all leaves occurred within the first day of evaluation for intact shoots stored at 0°C for 21 d. Due to involucral bract browning and collapse, vase life was terminated after 2 d for girdled shoots stored at 4.5°C for 21 d. A useable vase life of 6 d was obtained with girdled shoots stored at 0°C for 21 d (Fig. 9).

EFFECT OF COOLING METHOD ON POST-STORAGE VASE LIFE. A flowerhead core (or internal) temperature of 1°C was obtained with vacuum cooling within 20 minutes. The core temperatures of the flowerheads subjected to either 30 or 60 minutes of forced air cooling were 11.4 and 8.5°C, respectively. Vase life of vacuum cooled shoots was significantly extended to 8 d compared to forced air cooled shoots (5 d).

Discussion

Low temperatures were most successful in delaying leaf blackening in 'Sylvia' protea. Storage at 0°C resulted in a significantly lower number of blackened leaves for each treatment. The higher incidence of leaf blackening associated with increasing

storage temperature is in agreement with earlier reports (Jacobs & Minaar, 1977a). The significant increase in respiration rates with increasing temperature, which effectively depleted leaf carbohydrate content supported the earlier reports that increased respiration was correlated with leaf blackening (Ferreira, 1986). Furthermore, the higher respiration rate of the flowerhead confirms Ferreira's (1986) data and supports the hypothesis that flowerhead carbohydrate demands drive leaf blackening (Ferreira, 1986; Paull & Dai, 1990; Dai, 1993). Exposure to exogenous ethylene did not result in increased leaf blackening or shorter vase life (a specific flower response) in 'Sylvia' proteas. This is in agreement with earlier work that STS treatments did not suppress *P. eximia* leaf blackening (Newman *et al.*, 1990; Bieleski *et al*, 1992). In contrast to the climacteric respiration peak reported in *P. neriifolia* Huyser (1980), no climacteric was found in respiration of intact 'Sylvia' proteas, which may reflect genotypic variation. The decrease in respiration rate with time for temperatures greater than 0°C was possibly due to reduced carbohydrate availability for the flowerhead in order to maintain basal metabolism of leaves. The low carbohydrate content determined after three days of storage concurs with earlier reports that carbohydrate content of *Protea eximia* and *P. neriifolia* declined rapidly post-harvest (Bieleski *et al.*, 1992; McConchie & Lang, 1993). Suppression of leaf blackening in 'Sylvia' protea cut flowers held under light is in agreement with earlier reports on *P. cynaroides*, *P. eximia*, *P. repens*, *P. punctata* (Newman *et al.*, 1990) and *P. neriifolia* (Newman *et al.*, 1990; Paull & Dai, 1990; Jones & Clayton-Greene, 1992). Delaying the removal of field heat from 'Sylvia'

proteas was of greater importance than postharvest supplemental light when flowers were to be stored.

Some of the carbohydrate in leaves of *Protea* species susceptible to leaf blackening is bound with phenolics to form unstable *O*-glycoside esters (Perold *et al.*, 1973a,b; Perold *et al.*, 1979; Perold & Carlton, 1989, Perold, 1993). In contrast, phenolic compounds present in non-blackening Proteaceae members are in the form of stable *C*-glycosides (Elsworth & Martin, 1971; Perold, 1993). Perold (1993) hypothesised that the demand for carbohydrates for the developing flowerhead may stimulate or cause the cleavage of these compounds allowing the sugar(s) to be translocated to the flowerhead, and releasing a reactive phenolic moiety which can undergo non-enzymatic oxidation resulting in leaf blackening. The higher incidence and earlier onset of leaf blackening found in subtending flush leaves possibly reflects their role as a primary carbohydrate source for the flowerhead. Although no significant difference in leaf phenolic concentration was found between storage temperatures, the earlier onset and greater incidence of leaf blackening found with increasing storage temperature reflects an enhanced rate of phenolic oxidation.

The delay in onset of leaf blackening, caused by the removal of uppermost leaves of shoots subsequently stored for 3 d, may be attributed to a possible combination of factors. Carbohydrate depletion possibly occurs first in leaves immediately below the flowerhead due to their position and it is possible that leaves positioned further from the flowerhead have a higher initial carbohydrate concentration. Secondly, there is a possible delay in conversion of leaf carbohydrate reserves prior to translocation to the flowerhead as a consequence of the greater distance of the older flush leaves from the

flowerhead sink. It is possible that combination of these two factors results in a delay in the onset of the carbohydrate stress hypothesised to induce leaf blackening. The significant delay in onset of leaf blackening was more pronounced in shoots stored at 0°C emphasising the importance of low storage temperature on leaf blackening suppression.

The significant reduction in leaf blackening found in girdled 'Sylvia' proteas is in agreement with earlier reports which correlated flowerhead removal with a decrease in leaf blackening (Paull *et al.,* 1980; Brink & de Swardt, 1986; Brink, 1987; Reid *et al.*, 1989; Paull & Dai, 1990; Dai, 1993; Tranter, 1989; Dai & Paull, 1995). The over-riding factor of low temperatures during storage is demonstrated in girdled shoots stored for 21 d. Although girdling treatments significantly suppressed leaf blackening, flowerhead collapse in girdled shoots held at room temperature following 21 d at 4.5°C limited vase life to 2 d compared to that of 6 d for girdled shoots following 21 d storage at 0°C.

Vacuum cooling rapidly removes field heat (Thompson *et al.*, 1998) which is particularly important in *Protea* where the flowerhead bulk and overlapping involucral bracts impede field heat removal by forced air cooling.

Significant extension in transport longevity and post-storage vase life of 'Sylvia' protea cut flowers is possible when girdling directly beneath the flowerhead is used in combination with rapid removal of field heat and low storage temperatures.

Literature cited

Bieleski, R.L., Ripperda, J., Newman, J.P. & Reid, M.S., 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117, 124-127.

Brink, J.A., 1987. The influence of the flowerhead on leaf browning of cut *Protea neriifolia*. Protea News 5, 11-12.

Brink, J.A. & de Swardt, G.H., 1986. The effect of sucrose in vase solution on leaf browning of *Protea neriifolia* R. Br.. Acta Hort. 185, 111-119.

Dai, J-W., 1993. Postharvest leaf blackening in *Protea neriifolia* R. Br.. Ph.D. Diss., Univ. of Hawaii at Manoa, Honolulu.

Dai, J-W. & Paull, R.E., 1995. Source-sink relationship and *Protea* postharvest leaf blackening. J. Amer. Soc. Hort. Sci. 120, 475-480.

Elsworth, J.F. & Martin, K.R., 1971. Flavonoids of the Proteaceae, Part I. A chemical contribution to studies on the evolutionary relationships in the South African Proteoideae. J. S. Afr. Bot. 37, 199-212.

Faragher, J.D., Mayak, S., Tirosh, T. & Halevy, A.H., 1984. Cold storage of rose flowers: effect of cold storage and water loss on opening and vase life of 'Mercedes' roses. Scientia Hort. 24, 369-378.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescences. Acta Hort. 185, 121-129.

Gerber, A.I., Theron, K.I. & Jacobs, G., 2001. Manipulation of flowering time by pruning of *Protea* cv. Sylvia. HortScience 36, 909-912.

Goszczyñska, D.M. & Rudnicki, R.M., 1988. Storage of cut flowers. Hort Rev. 10, 35-62.

Haasbroek, F.J., Rousseau, G.G. & de Villiers, J.F., 1973. Effect of gamma rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5, 33-42.

Halevy, A. H. & Mayak, S., 1979. Senescence of postharvest physiology of cut flowers. Part 1. Hort. Rev. 1, 204-236.

Halevy, A. H. & Mayak, S., 1981. Senescence of postharvest physiology of cut flowers. Part 2. Hort. Rev. 3, 59-143.

Hardenburg, R.E., Watada, A.E. & Wang, C.Y., 1986. The commercial storage of fruits, vegetables, and florist and nursery stocks. USDA Agric. Handbook, 66 (Rev.), 75-92.

Huyser, C., 1980. Die invloed van fenoliese verbindings op sommige ensieme en metaboliese prosesse in loofblare van *Protea neriifolia* R. Br.. M.Sc. Thesis, Potchefstroom Univ. vir Christilike Hoër Onderwys, Potchefstroom, South Africa.

Ireland, J.P., Meynhardt, J.T. & Strauss, J.M., 1967. When proteas become sailors - treatment before shipping. Farming in South Africa 43, 33-35.

Jacobs, G., 1981. Postharvest handling of proteas. In: P. Mathews (Ed.), Growing and marketing of proteas, Vol. 1. Proteaflora Enterprises, Melbourne. pp 40-51.

Jacobs, G. & Minaar, H.R., 1977a. Effect of temperature on the blackening of *Protea* leaves. SAPPEX Newsletter 17, 20-24.

Jacobs, G. & Minaar, H.R., 1977b. Effect of light on the blackening of *Protea* leaves. SAPPEX Newsletter 18, 18-20.

Jones, R.B., 1991. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. Proc. Intl Protea Assoc. 6th Biennial Conf., Perth, Western Australia. pp 313-322.

Jones, R.B. & Faragher, J., 1991. Cold storage of selected members of the Proteaceae and Australian native cut flowers. HortScience 26, 1395-1397.

Jones, R.B. & Clayton-Greene, K.A., 1992. The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia* R. Br. leaves. Scientia Hort. 50, 137-145.

Levitt, J., 1972. Responses of plants to environmental stresses. Academic Press, New York. pp 27-43.

Lutz, J.M. & Hardenburg, R.E., 1968. The commercial storage of fruits, vegetables, and florist and nursery stocks. USDA Agric. Handbook 66 (Rev.), 55-87.

Mayak, S., 1985. Storage environment related stress and flower senescence. Acta Hort. 181, 33-43.

McConchie, R.B. & Lang, N.S., 1993. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118, 355-361.

McConchie, R.B., Lang, N.S. & Gross, K.C., 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116, 1019-1024.

McConchie, R.B., Lang, N.S., Lax, A.R. & Lang, G.A., 1994. Re-examining polyphenol oxidase, peroxidase and leaf blackening in *Protea*. J. Amer. Soc. Hort. Sci. 119, 1248-1254.

Meynhardt, J.T., 1976. Proteas - picking and handling. In: Farming in South Africa Flower, Ornamental Shrubs and Trees Series No. B5/1976. Dept Agric. Tech. Serv., Pretoria, South Africa.

Morris, L.L., 1969. A two stage, flow-through system for vegetables. Horticultural Report No. 9, Michigan State Univ., East Lansing, MI. pp 13-16.

Newman, J.P., van Doorn, W.G. & Reid, M.S., 1990. Carbohydrate stress causes leaf blackening in Proteas. Acta Hort. 264, 103-108.

Nowak, J., Rudnicki, R.M. & Duncan, A.A., 1990. Postharvest handling and storage of cut flowers, florist greens, and potted plants. Timber Press, Portland, Oregon.

Paull, R.E. & Dai, J-W., 1990. *Protea* postharvest blackleaf: a problem in search of a solution. Acta Hort. 264, 93-101.

Paull, R., Goo, T., Criley, R.A. & Parvin, P.E., 1980. Leaf blackening in cut *Protea eximia*: importance of water relations. Acta Hort. 113, 159-165.

Perold, G.W., 1993. Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. S. Afr. J. Sci. 89, 90-93.

Perold, G.W., Beylis, P. & Howard, A.S., 1973a. Metabolites of the Proteaceae. Part VII. Lacticolorin, a phenolic glucoside ester, and other metabolites of *Protea lacticolor* Salisb.. J. Chem. Soc. Perkin Trans I 1973, 638-642.

Perold, G.W., Beylis, P. & Howard, A.S., 1973b. Metabolites of Proteaceae. Part VIII. The occurrence of (+)-D-allose in nature: rubropilosin and pilorubrosin from *Protea rubropilosa* Beard. J. Chem. Soc. Perkin Trans I 1973, 643-649.

Perold, G.W., Rosenberg, M.E.K., Howard, A.S. & Huddle, P.A., 1979. Metabolites of Proteaceae. Part 9. Eximin (6-*O*-benzoylarbutin) and the synthesis of aryl glucoside ester. J. Chem. Soc. Perkin Trans I 1979, 239-243.

Perold, G.W. & Carlton, L., 1989. Neriifolin, an ester glucoside of 1,2,4-trihydroxybenzene. J. Chem. Soc. Perkin Trans I 1989, 1215-1217.

Reid, M.S., 1992. Postharvest handling systems: ornamental crops. In: A.A. Kader (Ed.), Postharvest technology of horticultural crops. Univ. of California, Oakland, CA. pp 201-209.

Reid, M.S. & Kofranek, A.M., 1980. Postharvest physiology of cut flowers. Chron. Hort. 20, 25-27.

Reid, M.S., van Doorn, W.G. & Newman, J.P., 1989. Leaf blackening in Proteas. Acta Hort. 261, 81-84.

SAPPEX, 2002. Export season July 2001-June 2002: *Protea* export figures. Internal report. South African Protea Producers and Exporters Association, South Africa.

Shelton, M.D., Walter, V., Brandt, D. & Mendez, V., 1996. The effects of refrigerated, controlled atmosphere storage during marine shipment on insect mortality and cut flower vase life. HortTechnology 6, 247-250.

Statistical Analysis Systems Institute, 1996. SAS® User's Guide, Version 6.12, Cary, NC.

Thompson, J.F., Mitchell, F.G., Rumsey, T.R., Kasmire, R.F. & Crisosto, C.H., 1998. Commercial cooling of fruits, vegetables and flowers. Univ. of California, Div. Agric. Nat. Res. Publ. 21567, Oakland, CA.

Tranter, D., 1989. Postharvest blackleaf in the *Protea* cultivar 'Pink Ice'. J. Intl Protea Assoc. 17, 13-20.

Van Doorn, W.G., 2001. Leaf blackening in *Protea* flowers: recent developments. Acta Hort. 545, 197-204.

Wand, S.J.E., 1995. Concentration of ultraviolet-B radiation absorbing compounds in leaves of a range of fynbos species. Vegtatio 116, 51-61.

Tables

Table 1.

Effect of supplemental postharvest photoperiodic light (12 h light/12 h dark) on the number of leaves with ≥10% blackened leaf area in 'Sylvia' protea cut flowers. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light for 10 d.

Light treatment	Day 2	Day 4	Day 6	Day 8	Day 10
12 h light/ 12 dark	2^{a}	3^{a}	5^{a}	5^{a}	6^{a}
Dark	3^{a}	5^{a}	8^{b}	13^{b}	16^{b}

Means (n=10) within columns followed by superscripts with the same letter are not statistically at $LSD_{(P=0.05)}$ level.

Table 2.

Total starch and reducing sugar content of 'Sylvia' protea subtending flush leaves at harvest and after 3 d storage at different temperatures.

Treatment	Total Starch (mg)	Total Reducing sugars (mg)
At harvest	140.85[a]	43.99[a]
Storage temperature for 3 d:		
0°C	129.02[a]	47.15[a]
4.5°C	83.56[b]	35.84[b]
7°C	77.89[c]	31.83[c]
10°C	57.03[d]	28.87[d]

Means (n=6) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 3.

Phenolic concentration of 'Sylvia' protea leaves from subtending and older flushes at harvest and after 3 d storage at different temperatures. Phenolic concentrations are expressed in terms of absorbance per gram dry mass determined at 280 nm ($A_{280} \cdot g^{-1}$), 300 nm ($A_{300} \cdot g^{-1}$) and 360 nm ($A_{360} \cdot g^{-1}$).

	Subtending flush			Older flushes		
	$A_{280} \cdot g^{-1}$	$A_{300} \cdot g^{-1}$	$A_{360} \cdot g^{-1}$	$A_{280} \cdot g^{-1}$	$A_{300} \cdot g^{-1}$	$A_{360} \cdot g^{-1}$
Harvest	4671[a]	4099[a]	1366[a]	4009[a]	3449[a]	1198[a]
Storage temperature for 3 d:						
0°C	5716[b]	4944[b]	1728[b]	4737[b]	3992[b]	1444[b]
4.5°C	5953[b]	5221[b]	1874[b]	4804[b]	4095[b]	1487[b]
7°C	6047[b]	5254[b]	1787[b]	4758[b]	4110[b]	1443[b]
10°C	6071[b]	5260[b]	1866[b]	5244[b]	4471[b]	1593[b]

Means (n=6) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 4.

Vase life of 'Sylvia' proteas that were either intact or where uppermost leaves were removed prior to 3, 7, 14 and 21 d storage at 0 and 4.5°C.

Treatment	Vase life (days)			
	3 d storage	7 d storage	14 d storage	21 d storage
0°C storage				
Intact	5^{b}	1^{a}	0^{a}	0^{a}
Leaves removed	8^{a}	2^{a}	0^{a}	0^{a}
4.5°C storage				
Intact	2^{b}	0^{a}	0^{a}	0^{a}
Leaves removed	5^{a}	0^{a}	0^{a}	0^{a}

Means (n=10) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Figures

Fig. 1. Terminology to describe *Protea* cut flowers. The most recently formed flush upon which the flowerhead is borne is termed the subtending flush. Older flushes comprised two vegetative flushes.

Fig. 2. Effect of supplemental postharvest photoperiodic light (110 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on the number of leaves with ≥10% blackened leaf area in 'Sylvia' protea cut flowers. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light. Data represented are the average of 10 shoots.

Fig. 3. Effect of storage temperature 0, 4.5, 7 and 10°C for 3 d on the subsequent leaf blackening (mean number of leaves with ≥10% blackened leaf area/shoot) of 'Sylvia' protea cut flowers held in water for 7 d. Data represented are the average of 60 shoots.

Fig. 4. Respiration rates [CO_2 evolution ($mL \cdot kg^{-1} \cdot h$)], of 'Sylvia' protea cut flowers determined after 12, 24, 48 and 72 h of storage at 0, 4.5, 7 and 10°C. Data represented are the average of 4 replications of 5 shoots.

Fig. 5. Respiration rates [CO_2 evolution ($mL \cdot kg^{-1} \cdot h$)] of intact, decapitated and leaf stripped 'Sylvia' protea cut flowers determined after 24, 48, 72 and 96 h of storage at 0°C. Data represented are the average of 4 replications of 5 shoots.

Fig. 6. Mean number of leaves, with at least 10% blackened leaf area, on shoots either kept intact or girdled directly beneath flowerhead. Shoots were stored at 0 and 4.5°C for 3, 7, 14 and 21 d prior to vase life assessment in a controlled temperature room (18±1°C) for 8 d. Data represented are the average of 60 shoots.

Fig. 7. Effect of storage temperature (0 and 4.5°C) on appearance of intact 'Sylvia' protea cut flowers after 10 d vase life assessment.

Fig. 8. Effect of storage temperature (0 and 4.5°C) and girdling treatment on appearance of 'Sylvia' protea cut flowers after 10 d vase life assessment.

Fig. 9. Effect of storage temperature (0 and 4.5°C) on appearance of girdled 'Sylvia' protea cut flowers after 21 d storage and 7 d vase life assessment.

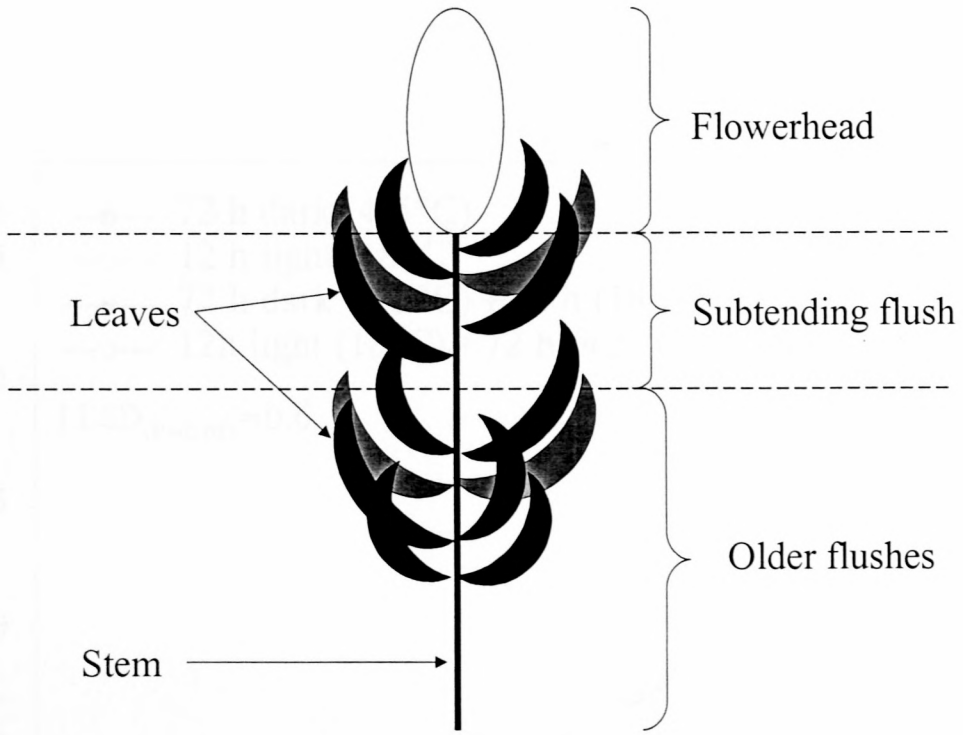


Fig. 1.

Terminology to describe *Protea* cut flowers. The most recently formed flush upon which the flowerhead is borne is termed the subtending flush. Older flushes comprised two vegetative flushes.

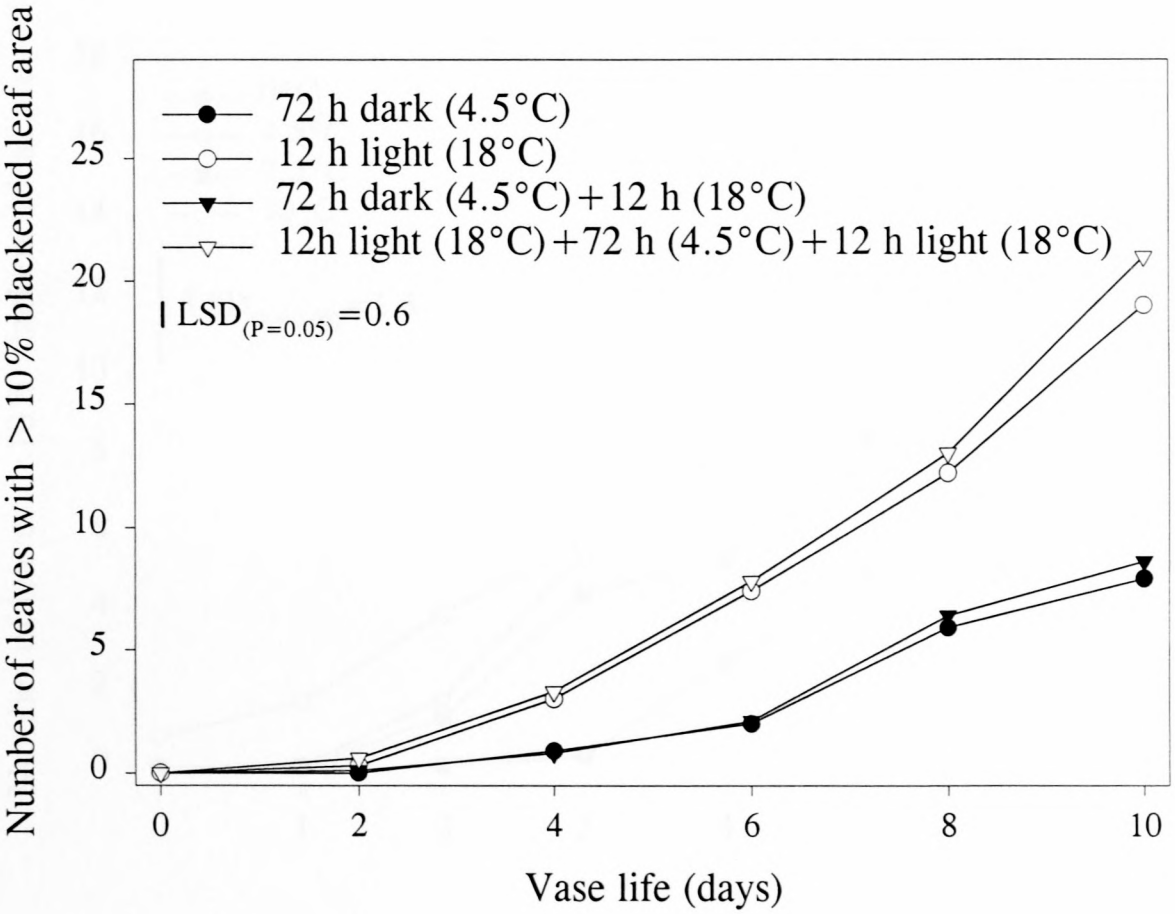


Fig. 2. Effect of supplemental postharvest photoperiodic light (110 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on the number of leaves with ≥10% blackened leaf area in 'Sylvia' protea cut flowers. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light. Data represented are the average of 10 shoots.

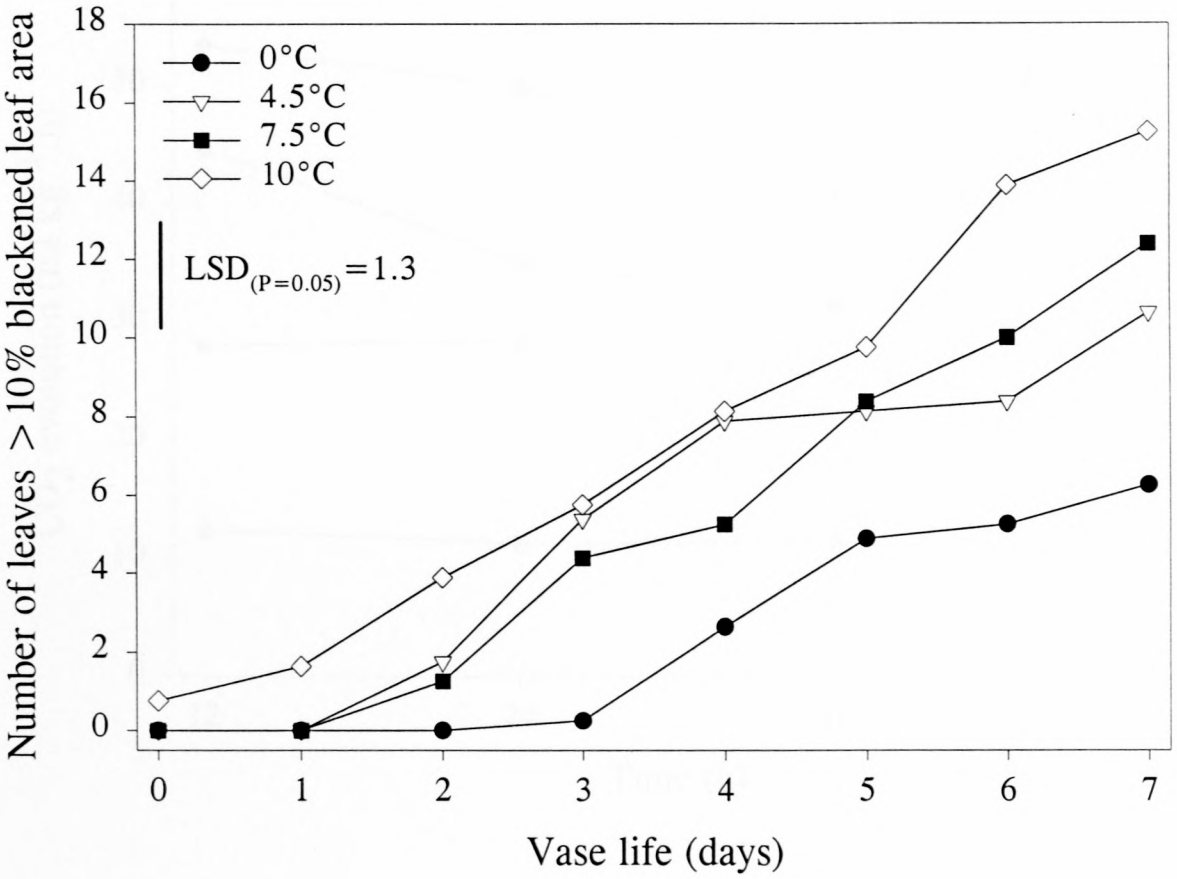


Fig. 3. Effect of storage temperature 0, 4.5, 7 and 10°C for 3 d on the subsequent leaf blackening (mean number of leaves with ≥10% blackened leaf area/shoot) of 'Sylvia' protea cut flowers held in water for 7 d. Data represented are the average of 60 shoots.

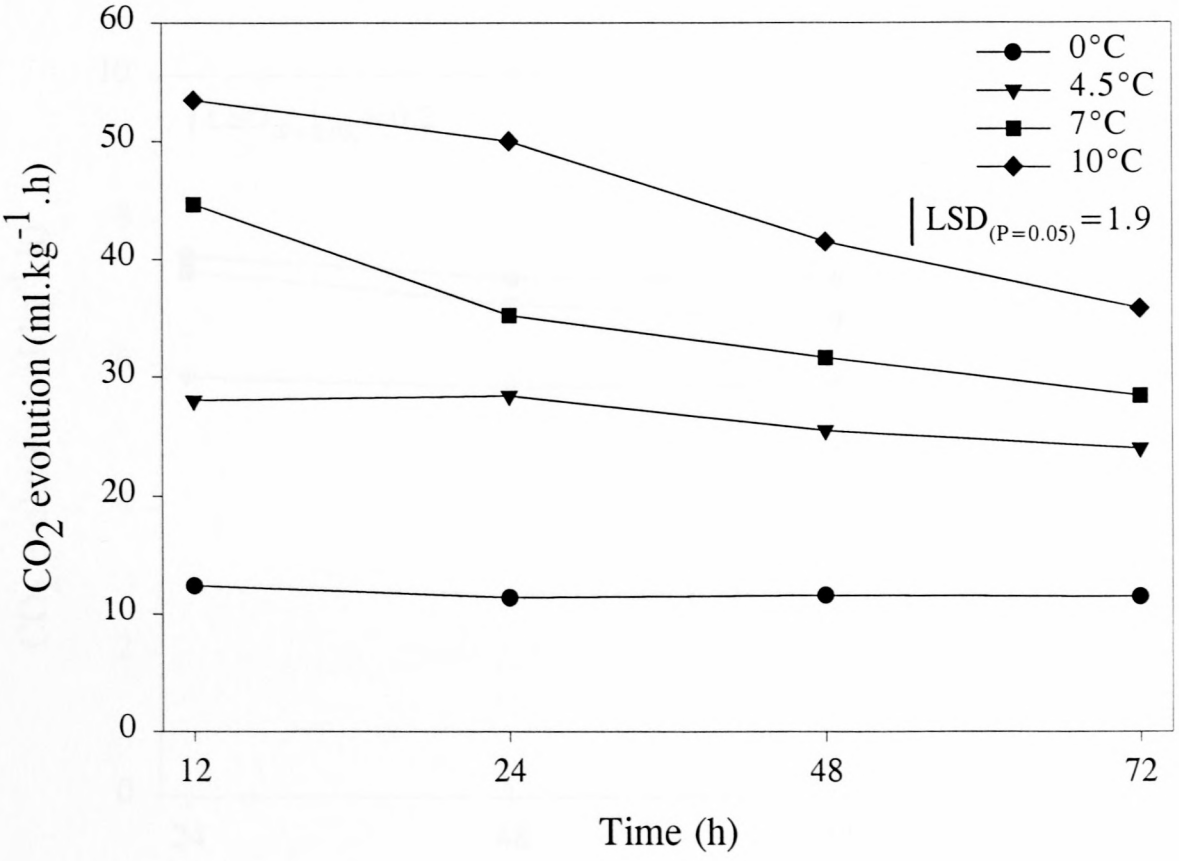


Fig. 4. Respiration rates [CO_2 evolution (mL·kg^{-1}·h)], of 'Sylvia' protea cut flowers determined after 12, 24, 48 and 72 h of storage at 0, 4.5, 7 and 10°C. Data represented are the average of 4 replications of 5 shoots.

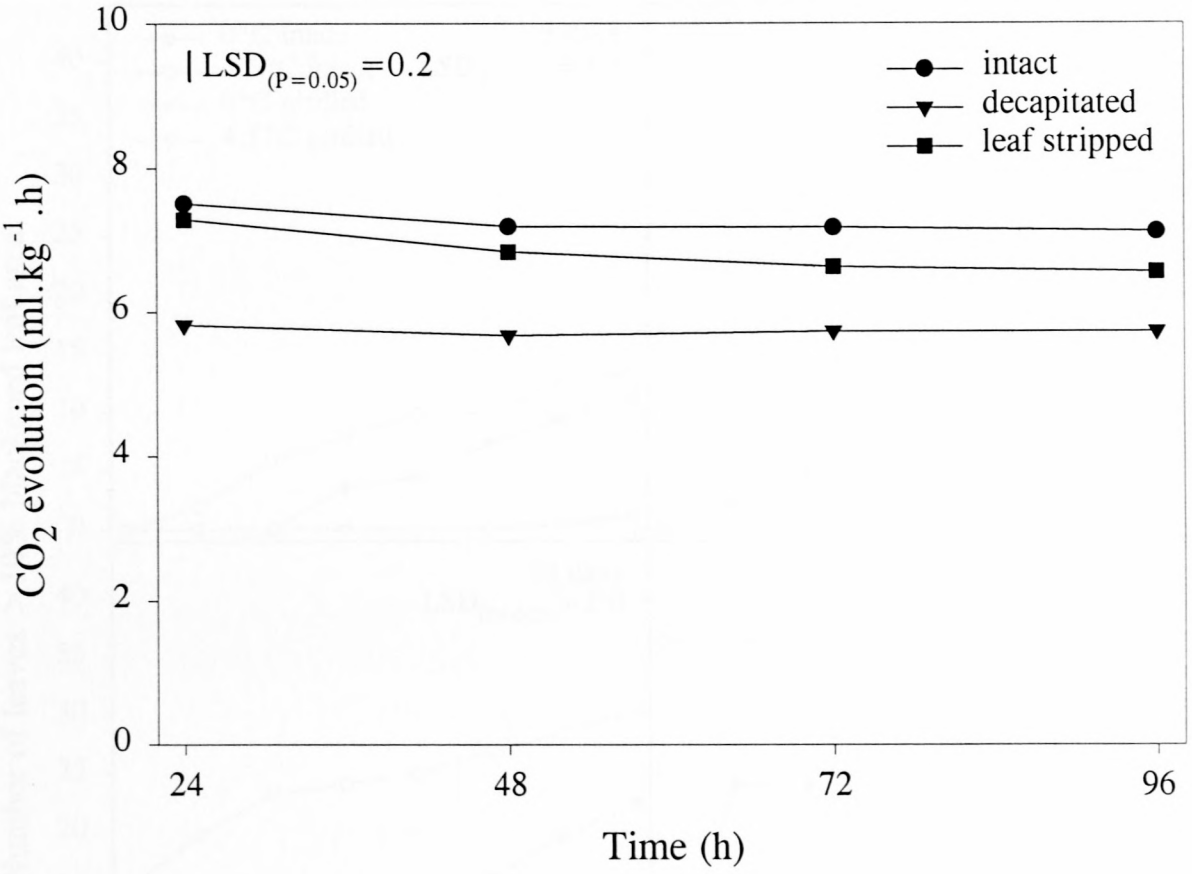


Fig. 5. Respiration rates [CO_2 evolution ($mL \cdot kg^{-1} \cdot h$)] of intact, decapitated and leaf stripped 'Sylvia' protea cut flowers determined after 12, 24, 48 and 96 h of storage at 0°C. Data represented are the average of 4 replications of 5 shoots.

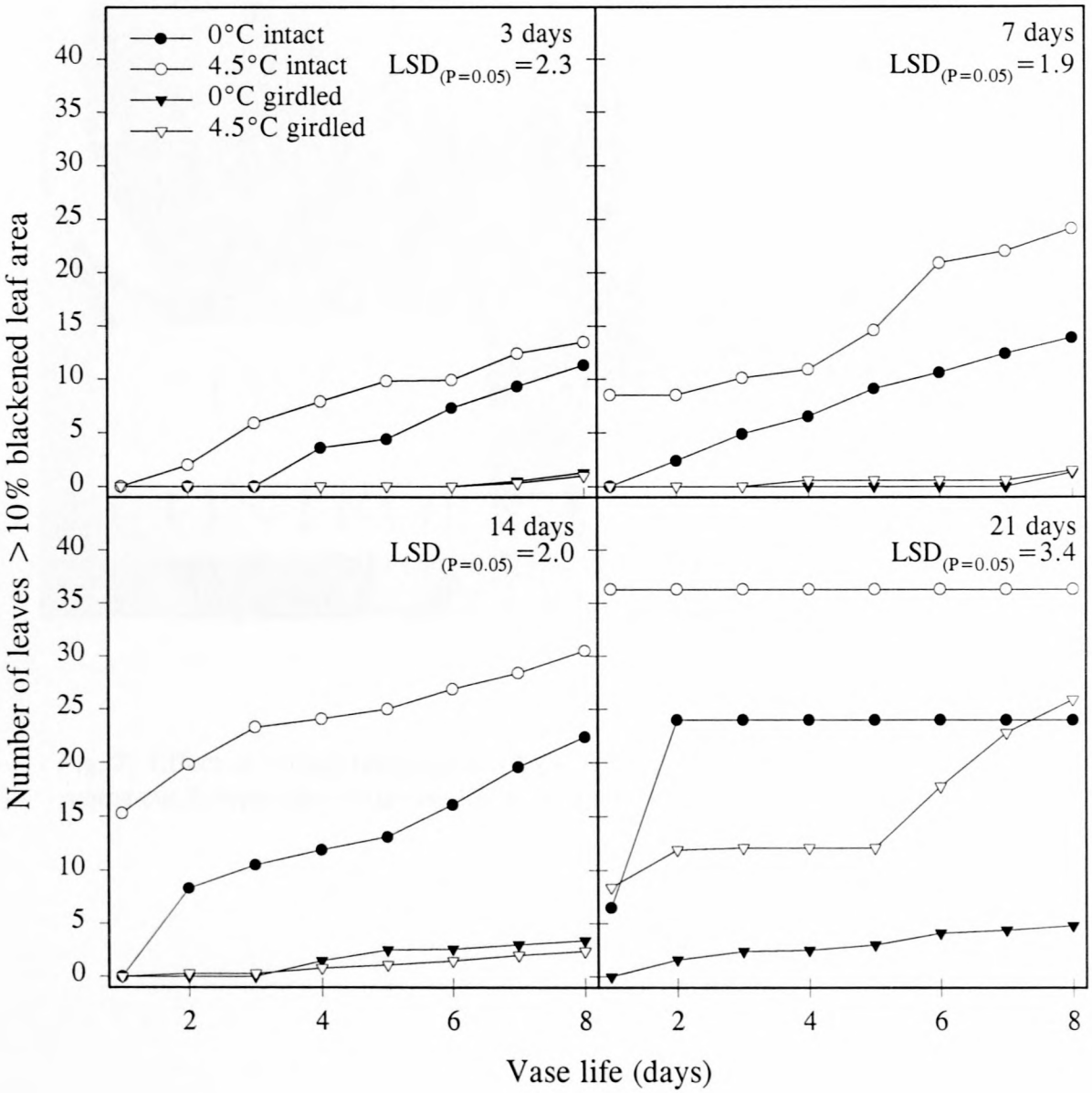


Fig. 6. Mean number of leaves, with at least 10% blackened leaf area, on shoots either kept intact or girdled directly beneath flowerhead. Shoots were stored at 0 and 4.5°C for 3, 7, 14 and 21 d prior to vase life assessment in a controlled temperature room (18±1°C) for 8 d. Data represented are the average of 60 shoots.

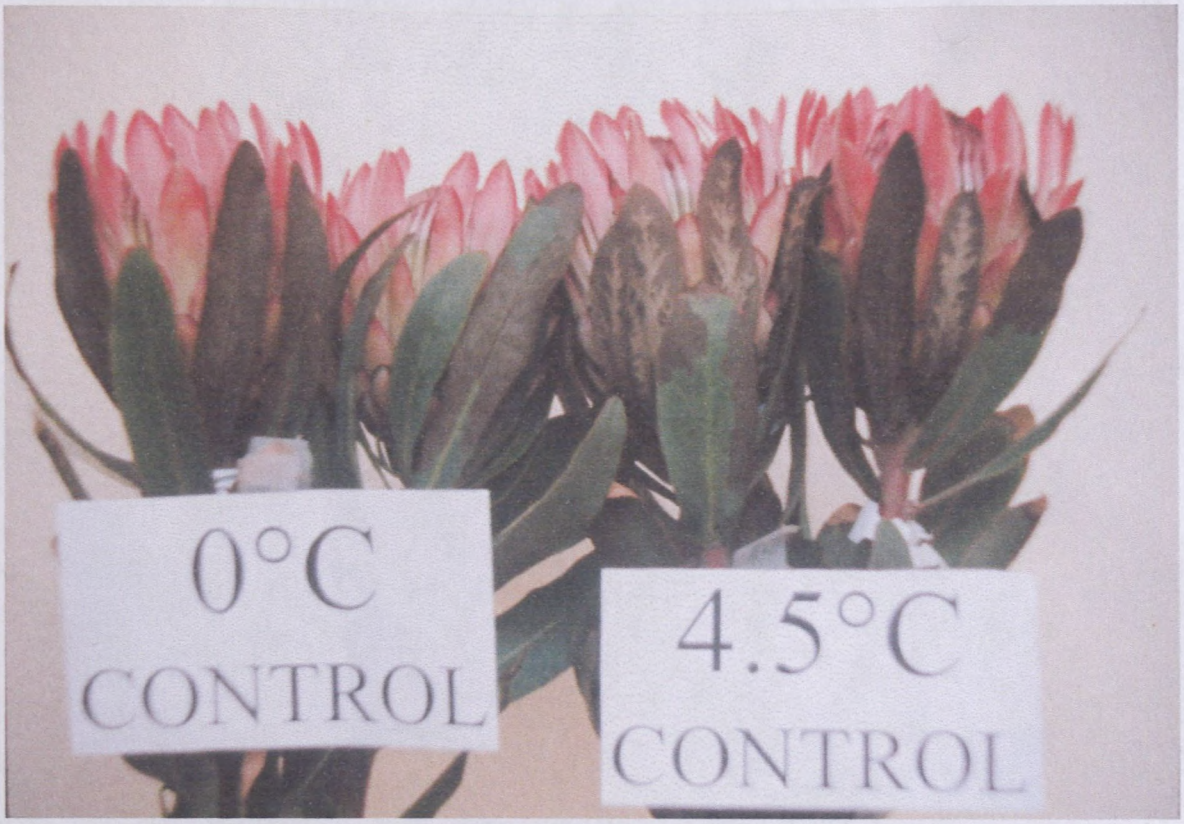


Fig. 7. Effect of storage temperature (0 and 4.5°C) on appearance of intact 'Sylvia' protea cut flowers after 10 d vase life assessment.

Fig.8. Effect of storage temperature (0 and 4.5°C) and girdling treatment on appearance of 'Sylvia' protea cut flowers after 10 d vase life assessment.

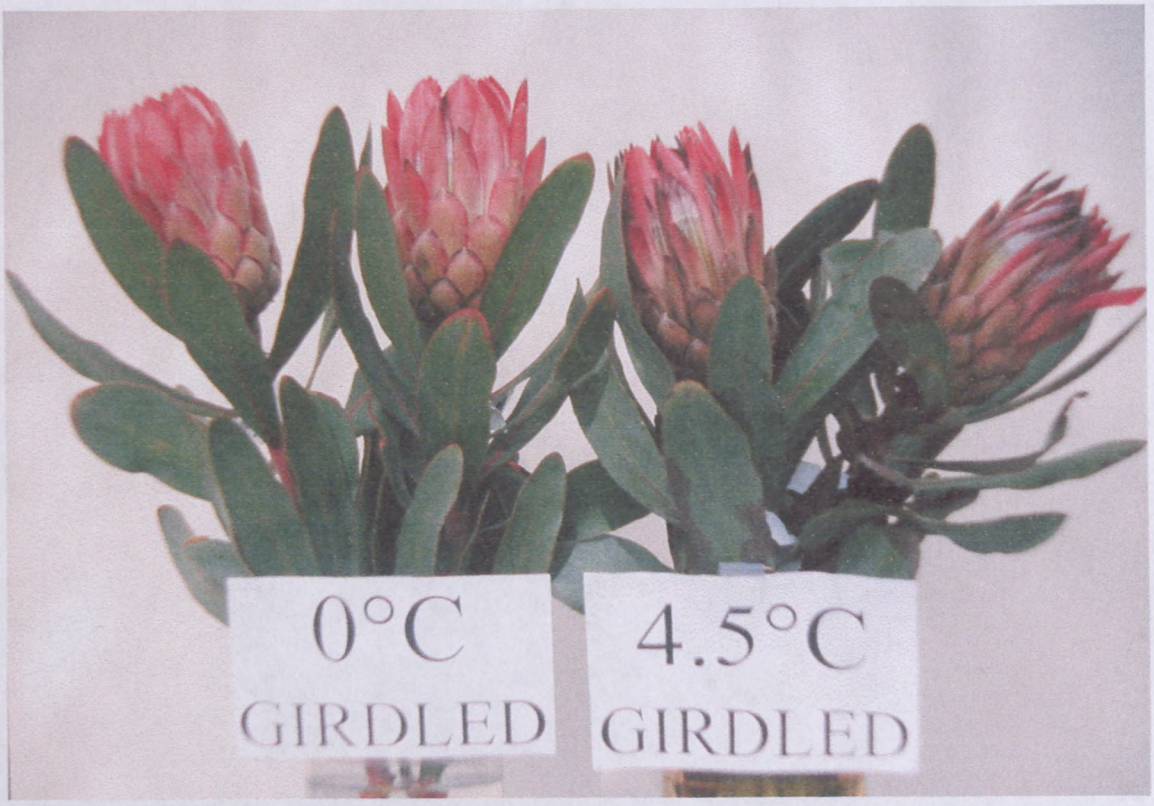


Fig. 9. Effect of storage temperature (0 and 4.5°C) on appearance of girdled 'Sylvia' protea cut flowers after 21 d storage and 7 d vase life assessment.

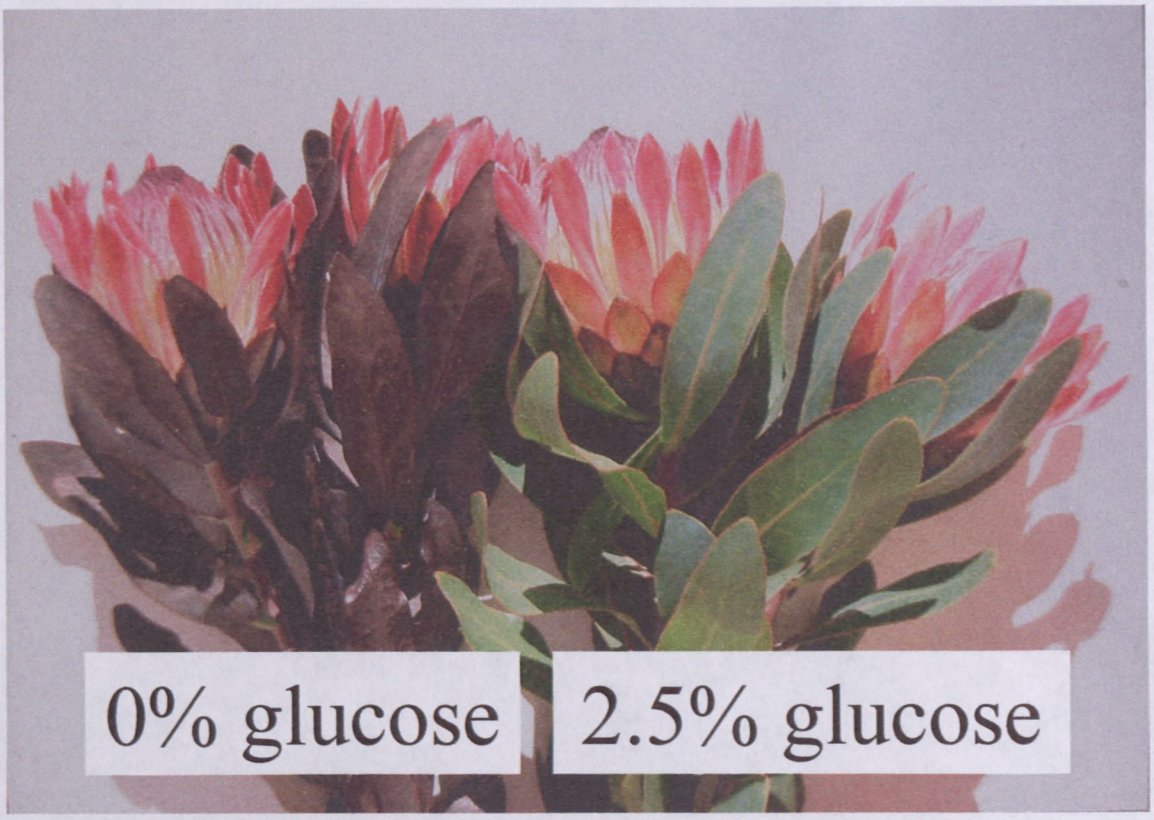


Fig. 2. Appearance of 'Sylvia' protea cut flowers subjected to 0 and 2.5% glucose holding solutions after 10 d vase life assessment.

3. PAPER II - Postharvest carbohydrate supplementation to extend 'Sylvia' protea vase life

Postharvest carbohydrate supplementation to extend 'Sylvia' protea vase life

Carbohydrate supplementation is a recognised practice in storage and vase life extension of many cut flower crops (Halevy & Mayak, 1974, 1979, 1981; Goszczyñska & Rudnicki, 1988; Nowak *et al.*, 1990). Supply of exogenous sucrose is reported to retard senescent processes in many cut flower crops by maintaining cellular membrane integrity and mitochondrial function, and delaying degradation of protein and ribonucleic acid (Halevy & Mayak, 1979; Nowak *et al.*, 1990).

Utilisation of exogenous sugars in *Protea* cut flowers has only been partially successful. Sucrose holding solutions (≤ 2 g·L^{-1}) have been reported to effectively reduce postharvest leaf blackening of *P. compacta* (Ireland *et al.*, 1967; Haasbroek *et al.*, 1973), *P. eximia* (Ireland *et al.*, 1967; Bieleski *et al.*, 1992), *P. cynaroides* and *P. magnifica* (Ireland *et al.*, 1967), and *P. neriifolia* (Mulder, 1977; Brink & de Swardt, 1986; Brink, 1987; Paull & Dai, 1990; McConchie *et al.*, 1991). Holding solutions with sucrose at higher concentrations exacerbated *P. neriifolia* leaf blackening (Brink, 1987; Jones 1991a). In contrast a 30 g·L^{-1} sucrose holding solution significantly suppressed leaf blackening of *P. eximia* (Akamine *et al.*, 1979). Sucrose pulsing solutions (200 g·L^{-1}, 24 h, 1°C) significantly reduced leaf blackening in *P. cynaroides* during long term storage at 1°C (Jones, 1991a) as did sucrose pulsing (200 g·L^{-1}, 24 h,

25°C) of *P. neriifolia* during 7 d dark, wet storage at 25°C (McConchie & Lang, 1993).

Sucrose supplementation in several other Proteaceae genera has met with mixed success. Holding solutions which incorporated sucrose significantly extended vase life of *Banksia speciosa* (Parvin, 1978), *Banksia prionotes* (Faragher, 1989), *Grevillea* spp. (Lacey, 1982; Faragher, 1989; Vuthapanich *et al.*, 1994; Ligawa *et al.*, 1997), *Leucadendron* cv. Silvan Red (Jones, 1991b), *Leucospermum cordifolium* (Parvin, 1978; Criley *et al.*, 1979), *Leucospermum nutans* and *Leucospermum lineare* (Ireland *et al.*, 1967) and *Serruria florida* (Ireland *et al.*, 1967).

Leaf blackening induced by gamma radiation was inhibited by a 3.5% sucrose solution, indicating a possible reduction in cellular membrane damage caused by irradiation (Haasbroek *et al.*, 1973). It has been hypothesised that water loss from leaves, brought about by flowerhead transpiration, effects a water stress which damages cell membranes thereby contributing to leaf blackening (de Swardt, 1979; Paull *et al.*, 1980; Ferreira, 1983; de Swardt *et al.*, 1987; Paull & Dai, 1990). Although decreasing flowerhead transpiration and subjecting shoots to a period of water stress had no effect on *P. eximia* leaf blackening (Reid *et al.*, 1989) the application of anti-transpirants to *P. neriifolia* leaves significantly reduced leaf blackening (Paull & Dai, 1990).

Use of postharvest supplemental photosynthetic light to increase carbohydrate levels had no significant effect, but it did have an effect on 'Sylvia' protea vase life

(Paper I). However, use of light during pulse treatments is recommended to prolong both flower quality and longevity (Halevy & Mayak, 1981; Nowak *et al.*, 1990).

Over the past five years South African exporters and producers of cut flowers have had to contend with a significant increase in both cost and unavailability of airfreight space to European markets. This directed research into the possibility of shipping proteas by sea, which requires 21 d from the time of product delivery to the local exporter to product arrival in Europe. It was hypothesised that exogenous sugar supplementation would extend vase life of 'Sylvia' protea cut flowers. Analysis of the sugar content of both flowerhead and leaves was undertaken when no beneficial response to sucrose holding or pulsing solutions was found. Based on these results addition of glucose, instead of sucrose, to holding solution and pulsing solutions was tested on 'Sylvia' proteas. In conjunction with vacuum cooling and low storage temperature (Paper I) the effect of time of harvest, pulsing conditions (temperature and light) and methods to limit possible detrimental effects of water loss during 21 d dry storage of 'Sylvia' protea cut flowers were investigated.

Materials and Methods

PLANT MATERIAL. Flower-bearing 'Sylvia' protea shoots were harvested from a commercial plantation, Protea Heights, located in the Stellenbosch district, (33°55'S; 18°50'E), South Africa. The area has a Mediterranean climate with hot, dry summers and a rainfall of 600-700 mm falling mainly in winter. Shoots were harvested into water and brought to the laboratory within an hour of harvest, cut to 55 cm in length

and the bottom 20 cm stripped of leaves. Vase life was assessed in a controlled temperature room (18±1°C) subject to natural light.

SUCROSE HOLDING AND PULSING SOLUTIONS. Shoots were placed in individual vases containing 1, 2 or 5% sucrose solutions. Shoots placed in distilled water served as control. Other shoots were pulsed for 12 h (18±1°C) with 0, 10 or 20% sucrose solutions. The shoots were then held for 8 h at 0°C and then packed into SAPPEX S14 fibreboard cartons with lids. Cartons were stored at 0°C for 3 d, after which stems were recut to 50 cm and placed in individual vases containing distilled water and vase life assessed for 8 d. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Ten single shoot replicates per treatment were used.

HPLC ANALYSIS OF INDIVIDUAL SUGARS. Shoots were stripped of all but subtending flush leaves, placed in tap water and cooled for 8 h at 0°C. Shoots were packed into SAPPEX S14 cartons with lids and stored for 3 d at 0°C in darkness, before being recut to 50 cm and placed in individual vases containing tap water and vase life assessed for 6 d.

At harvest, after 3 d storage at 0°C, and after 2 and 6 d at 18±1°C, flowerhead, leaves and stems were separated, lyophilised and dry mass determined, before being milled to a fine powder. HPLC analysis of individual sugars was based on the method described by Perez *et al.* (1997). Samples were extracted for 1 h in 25 mL of 95% ethanol by shaking and then centrifuged (10,000 g_n, 20 min, 4°C). Supernatant was

collected and the pellet resuspended in 10 mL of 80% ethanol and centrifuged (10,000 g_n, 20 min, 4°C). Supernatant fractions were combined and made up to 50 mL with 80% ethanol. An aliquot of 10 mL was taken and evaporated to dryness on a vacuum centrifuge (model SVC200H; Savant, Farmingdale, NY). Residue was dissolved in 2 mL of 0.2N H_2SO_4 and filtered through a 0.5 μm filter. Sample was washed through with a further 2 mL of 0.2N H_2SO_4 then loaded onto an activated Sep-Pak® C_{18} cartridge (Waters, Milford, MA) and the eluate collected. Sample was washed through the activated cartridge with a further 2 mL of 0.2N H_2SO_4. Sugars were separated using an HPLC system (1100 Series; Hewlett Packard, Waldbronn, Germany) with an autosampler (1100 Series; Hewlett Packard) operated by HP ChemStation software (LC Rev.A.06.03 [509]; Hewlett Packard). A Transgenomic™ ion exchange stainless steel column (300 x 7.8 mm) (model ICSep ICE-ION-300; Transgenomic, Omaha, NE) with a guard column (model GC-801; Transgenomic) was maintained at 30°C. Sugars were separated using 0.0085N H_2SO_4 at a flow rate of 0.5 $mL \cdot min^{-1}$. A differential refractive index monitor (model R401; Waters) was used to detect separated sugars. An injection volume of 30 μL per sample was used. Six single shoot replicates per treatment were used.

GLUCOSE HOLDING SOLUTION. Shoots with leaves stripped from the bottom 20 cm were placed into individual vases containing 0, 0.025, 0.25 or 2.5% glucose solution. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning

≥20% and involucral bract collapse ≥10% was deemed unacceptable. Ten single shoot replicates per treatment were used.

GLUCOSE PULSING SOLUTION. Shoots with leaves stripped from the bottom 20 cm were placed into individual vases containing 0 (control), 1, 2, 3, 4, 5, 6, 8 or 10% glucose solution at 18±1°C for 24 h. After pulsing flowers were placed into individual vases containing tap water and vase life assessed for 14 d. The number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Ten single shoot replicates per treatment were used.

EFFECT OF TIME OF HARVEST, PULSING TEMPERATURE AND LIGHT ON PULSE SOLUTION UPTAKE. Shoots were harvested in the morning (07h00; 10°C) and afternoon (15h00; 30°C) and brought to the laboratory within 1 h of harvest. Shoots were placed into individual 100 mL measuring cylinders containing either 0 (control) or 5% glucose solutions inside growth chambers (model Economic Delux; Snijders Scientific, Holland) maintained at 20, 25 and 30°C. Photosynthetic lighting was supplied at a constant rate of 140 $\mu mol \cdot m^{-2}\ s^{-1}$ by 14 photosynthetic lighting tubes (model TL Tube 36W; Type Brite Gro 2084; Sylvania, Germany). Growth chambers with no supplemental lighting served as control. Time to take up 10 mL of 5% glucose solution was determined. A total of 500 mg glucose was taken up by each shoot. Shoots were packed into SAPPEX S14 fibreboard mini-cartons, vacuum cooled (1°C), then stored for 21 d (1°C, 95% RH). Vase life was assessed for 10 d using the

number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for vase life termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Ten single shoot replicates per treatment were used.

PACKAGING. Shoot mass was determined directly after pulsing with either 0 (control) or 5% glucose solution (10 mL, 25°C, 140 $\mu mol \cdot m^{-2} s^{-1}$). Shoots were then packed into SAPPEX S14 fibreboard mini-cartons lined with polyethylene (PE) sheets (25 μm thickness) and vacuum cooled to 1°C. Unlined cartons served as control. Lined cartons were sealed inside PE bags (25 μm thickness) and, along with control cartons, stored for 21 d (1°C, 95% RH). At one week intervals gas samples were drawn from each carton and CO_2 and O_2 content determined by gas chromatography (model HP 5890, Series 2; Hewlett Packard). After 21 d storage shoot mass and % mass lost per shoot were determined. Vase life was assessed for 8 d. Number of leaves with at least 10% leaf area blackened was determined daily. Two replications of ten single shoots per treatment were used.

CARBOHYDRATE ANALYSES. Upon completion of pulse treatments with 0 (control) or 5% glucose solution (10 mL, 25°C, 140 $\mu mol \cdot m^{-2} s^{-1}$) and after 21 d storage (1°C, 95% RH) inside PE lined and enclosed SAPPEX S14 fibreboard mini-cartons, shoots were separated into flowerhead, leaf and stem components. Samples were lyophilised and dry mass determined, before being milled to a fine powder. A 0.5 g sample of the dried tissue described was taken for glucose and starch analysis. Samples were

extracted for 18 h in 5% acetic acid by shaking and centrifuged (4,000 g_n, 10 min). Supernatant was filtered and made up to 100 mL with 5% acetic acid. Thereafter the pellet was resuspended in an acetate buffer (pH 4.8) and gelatinised in a boiling steam bath for 2 h. The suspension was cooled to 60°C and the starch fraction hydrolysed to glucose with amyloglucosidase (EC 3.2.1.3) (Fluka Chemie, Buchs, Switzerland). Hydrolysis was performed in an incubator maintained at 55°C for 18 h. Analysis of glucose and starch was done on a Sanplus Segmented Flow Analysis System (method numbers 551-965w/r issue 070798/MH and 356-001w/r issue 012998/MH97203066, Skalar, De Breda, The Netherlands). Twelve single shoot replicates per treatment were used.

STATISTICAL ANALYSIS. Standard analysis of variance was performed using the General Linear Model procedure generated by the SAS® program (Statistical Analysis Systems Institute, 1996). Students t-LSD were calculated at a 5% level to compare treatment means.

Results

SUCROSE HOLDING AND PULSING SOLUTIONS. Sucrose holding solutions (1%) did not significantly improve 'Sylvia' protea vase life whilst 2 and 5% sucrose solutions promoted leaf blackening (Table 1). Similarly, pulsing with sucrose (10 and 20%) did not significantly improve vase life of 'Sylvia' proteas (Table 1).

HPLC ANALYSIS OF INDIVIDUAL SUGARS. Analysis of sugars (Table 2) demonstrated a significant decrease in leaf glucose and sucrose concentration during

storage (3 d at 0°C). There was a significant increase in leaf sucrose concentration and concurrent increase in flowerhead sucrose concentration during the first 2 d of vase life. Glucose and fructose concentrations were significantly higher than those of sucrose in both leaves and flowerhead. Flowerhead fructose concentration did not change significantly during the experiment, whilst leaf fructose concentration decreased significantly only after removal from storage. There was a consistent decrease in leaf and flowerhead glucose concentration during storage and vase life.

GLUCOSE HOLDING SOLUTION. A holding solution of 2.5% glucose significantly reduced leaf blackening in 'Sylvia' proteas (Figs 1 & 2). Shoots held in water and the lower glucose concentrations blackened rapidly and vase life evaluation was terminated after 10 d. 'Sylvia' proteas held in 2.5% glucose solutions showed minimal blackening even after 20 d at 18±1°C, where vase life was terminated due to flowerhead collapse and not leaf blackening.

GLUCOSE PULSING SOLUTION. A vase life of 14 d was obtained in shoots pulsed with either 5 or 6% glucose solutions (Table 3). This represented a significant vase life extension of 9 d over control shoots. Involucral bract discolouration and collapse in shoots pulsed with 8 and 10% glucose solutions limited vase life to 11 and 12 d respectively. In shoots pulsed with 10% glucose browning and collapse of involucral bracts was accompanied by the crystallisation of sugar on involucral bract surfaces. No significant difference in vase life was found between 0 (control), 1 and 2% glucose pulsing solutions.

EFFECT OF TIME OF HARVEST, PULSING TEMPERATURE AND LIGHT ON SOLUTION UPTAKE. Supplemental photosynthetic lighting significantly reduced uptake time for both harvest times, although the reduction was of greater significance in shoots harvested in the morning (Table 4). Increasing pulse temperature resulted in a significant decrease in uptake time in shoots harvested in the afternoon. Uptake time also decreased significantly with increasing temperature in shoots harvested in the morning and pulsed under photosynthetic light. The uptake rate of morning-harvested shoots pulsed in the dark was significantly faster when pulsing was performed at 25°C.

Vase life was significantly reduced in shoots harvested in the morning and subjected to a dark pulse treatment (Table 5), compared to those shoots pulsed in the light. Although significant differences in absolute vase life values were found between remaining treatments these were of no practical significance.

PACKAGING. There was a significantly smaller loss in fresh mass (%) in shoots packed into PE lined and enclosed cartons during storage (21 d, 1°C, 95% RH) than in those control shoots packed into unlined, unenclosed cartons (Table 6). Leaf blackening incidence was significantly lower in shoots pulsed with 5% glucose solution (Fig. 3). Non-pulsed control shoots had a significantly lower incidence of leaf blackening when stored in PE lined, enclosed cartons than non-pulsed shoots stored in unlined cartons after 5 d vase life (Fig. 3). However, differences were not significant for all other evaluation dates. This difference was not found in shoots pulsed with 5% glucose. Analysis of gas samples drawn from sealed cartons did not differ significantly from ambient atmosphere during 21 d storage (data not presented).

CARBOHYDRATE ANALYSES. Mean total starch content of leaves determined upon completion of pulsing treatments was significantly greater in shoots pulsed with 5% glucose solutions (174.0 mg) than that of non-pulsed control (93.8 mg) (Fig. 4). No significant difference in total starch content of flowerhead and stem tissues was found between treatments. There was a significant decrease in mean total starch content for all tissues during 21 d storage although no significant difference between pulse treatments was found. Mean total glucose content, determined upon completion of pulsing treatments, was significantly greater in flowerhead tissue (565.8 mg) of shoots pulsed with 5% glucose solutions compared with non-pulsed control shoots (470.9 mg) (Fig. 5). Mean total glucose content of leaves (109.1 mg) and stems (97.5 mg) of shoots pulsed with 5% glucose solutions was significantly greater than that of the non-pulsed control leaves (57.3 mg) and stems (19.6 mg) respectively when determined upon completion of pulsing treatments. There was a significantly greater mean total glucose content in leaves in shoots pulsed with 5% glucose (45.8 mg) than non-pulsed control shoots (11.1 mg) after storage. No significant difference in mean total glucose content of flowerhead and stem tissues between pulse treatment was found after 21 d storage.

Discussion

Susceptibility to leaf blackening ranges widely between species (McConchie & Lang, 1993), selections within a species (Mulder, 1977; Paull & Dai, 1990) and time

of year (de Swardt *et al.*, 1987) and it is perhaps therefore not surprising that a wide range of response to sucrose supplementation exists within *Protea* cut flowers.

In contrast to the significant vase life extension by 1% sucrose holding solutions of *P. eximia*, a parent of 'Sylvia' (Bieleski *et al.*, 1992), and the beneficial effects of sucrose pulse solutions in *P. neriifolia* (McConchie & Lang, 1993) no benefit was found in sucrose supplementation for 'Sylvia' proteas. These results may reflect genotypic variation and indicate the importance of evaluating treatments at a cultivar level.

The significant decrease in leaf sucrose during storage is thought to reflect maintenance of both leaf and flowerhead basal metabolism. The increase in sucrose concentration in leaves, subsequent to storage and 2 d vase life, is thought to reflect mobilisation of stored carbohydrate reserves and translocation to the flowerhead to meet respiratory demand and nectar production. This is supported by the significant concurrent increase in flowerhead sucrose concentration. The subsequent decrease in flowerhead sucrose concentration is attributed to ongoing flowerhead respiratory demands during vase life assessment. Analyses indicated that fructose remained unchanged in the flowerhead through storage and vase life evaluation whilst that of leaves decreased subsequent to storage removal. In contrast, glucose decreased consistently in leaves and flowerhead in storage and during vase life and was the reason for addition of glucose, in place of sucrose, to holding solutions. Furthermore, addition of fructose to holding solutions had no effect on leaf blackening (Gerber, pers. comm.).

The significant 10 d extension in vase life of 'Sylvia' proteas held in 2.5% glucose solutions was due to a significant reduction in leaf blackening. Vase life was terminated due to flowerhead collapse instead of leaf blackening for the first time in 'Sylvia' protea cut flowers. At present it is uncertain as to why supplying glucose, instead of sucrose, in both pulse and holding solutions improved 'Sylvia' protea vase life. That sucrose was ineffective in suppression of 'Sylvia' protea leaf blackening may reflect a limitation in either activity or quantity of invertase, which cleaves sucrose into glucose and fructose; sucrose synthase, which, in the presence of UDP, converts sucrose to UDP-glucose and fructose, or a combination of these two enzymes. A further possibility may be that there are a limited number of sucrose transporters in 'Sylvia' proteas, which would preclude increased uptake. Several monosaccharide transporters have been identified in a variety of plant species (Maynard & Lucas, 1982; Getz *et al.*, 1987; Gogarten & Bentrup, 1989; Tubbe & Buckhout, 1992). It is possible that glucose transport in 'Sylvia' proteas is similar to that found in *Saccharomyces cerevisiae* (wild yeast) (Özcan & Johnston, 1995; Bolles & Hollenberg, 1997) and regulated at the transcriptional level in response to external glucose concentration. It is possible that increased external glucose concentrations elicited an increased transcription of a hexose transporter, e.g. HXT1, a low affinity glucose transporter induced only by high glucose concentrations (Özcan & Johnston, 1995; Bolles & Hollenberg, 1997), which facilitated an increased glucose uptake.

Nectar production has been hypothesised to cause carbohydrate depletion in leaves and positively correlated with leaf blackening (Paull & Dai, 1990; Dai, 1993). *Protea*

species produce significant nectar volumes (Mostert *et al.*, 1980; Cowling & Mitchell, 1981; Wiens *et al.*, 1983) of which glucose has been identified as a major component (Cowling & Mitchell, 1981; Van Wyk & Nicholson, 1995). In *P. eximia* and *P. susannae*, parents of *Protea* cv. Sylvia, glucose comprised >50% and >35% of the total nectar sugars respectively, whilst sucrose comprised 2% and ≤27% of total nectar sugars in *P. eximia* and *P. susannae* respectively (Van Wyk & Nicholson, 1995). Cowling & Mitchell (1981) reported that >30% of ^{14}C applied to *P. neriifolia* was located in the nectar within 24 h. Dai (1993) reported >50% of ^{14}C applied to be located in the nectar within the same time frame. Perold (1993) hypothesised that carbohydrate demands by the developing flowerhead stimulated or caused the cleavage of the carbohydrate bound in formation of phenolic esters to release a reactive phenolic moiety which could undergo non-enzymatic oxidation resulting in leaf blackening. Flowerhead appearance (involucral bract browning and collapse) was not significantly affected by girdling (Paper I). It is possible that glucose supplementation enabled nectar production to proceed without the depletion of leaf carbohydrate thereby contributing to the suppression of leaf blackening in 'Sylvia' proteas.

Although glucose holding solutions provided a breakthrough in suppression of leaf blackening in 'Sylvia' protea cut flowers these are impractical for use during storage and transport. In order to determine a practical commercial application for this research the use of glucose pulsing solutions was investigated. Vase life of all shoots was significantly extended with ≥3% glucose pulse solutions. Leaf blackening was significantly suppressed with increasing glucose pulse concentration. However,

pulsing with ≥8% glucose solutions resulted in suppression of beneficial vase life extension due to browning and collapse of involucral bracts. This browning and collapse was accompanied by the appearance of sugar on the bract surfaces and was attributed to toxicity. The fact that 1 and 2% glucose solutions did not significantly extend vase life indicated that this level of supplementation was inadequate for leaf blackening prevention.

It is thought that placing shoots harvested in the morning under high intensity PAR stimulated photosynthesis, increased stomata opening consequently increasing both transpiration and rate of glucose solution uptake, to a greater extent than in shoots harvested in the afternoon. The faster uptake of glucose solutions in shoots harvested in the afternoon was attributed to higher shoot temperatures and consequent transpiration rate compared to those harvested in the morning. In shoots harvested in the afternoon, irrespective of light treatment, there was a decrease in uptake time with increasing temperature, which was attributed to increased transpiration rate. Uptake time in shoots harvested in the morning and pulsed in the dark was shortest when done at 25°C. It is possible that in the 30°C, dark pulsing treatment the stomata closed in order to minimise water loss.

The significant increase in vase life of shoots harvested in the morning and pulsed under PAR light was attributed to the faster uptake of glucose solutions which enabled vacuum cooling to be performed earlier than that of shoots pulsed in the dark. This lends impetus for cooling *Protea* cut flowers as soon as possible post-harvest in order to facilitate carbohydrate conservation and benefit storage and vase life extension.

Leaf blackening was significantly lower in non-pulsed shoots packed into lined and enclosed cartons, compared to non-pulsed shoots packed into unlined, unenclosed cartons, indicating that water loss does have an influence on *Protea* cut flowers. However, this was for only one of the eight evaluation dates and was of no practical significance. However, the most important factor in leaf blackening suppression was determined to be glucose supplementation. In shoots pulsed with 5% glucose solutions no significant difference in leaf blackening was found between packaging treatments. The significant reduction in fresh mass loss in shoots pulsed with 5% glucose solutions concurs with Jones (1991b) who reported that *Leucadendron* leaves on shoots subjected to a 200 $g \cdot L^{-1}$ sucrose pulse (24 h, 1°C) were protected from desiccation during 42 d storage at 1°C. These findings are in agreement with the hypothesis that sugars, as well as supplying carbohydrate for respiration, are also involved in cell membrane protection (Santarius, 1973; Halevy & Mayak, 1979; Jones *et al.*, 1995).

Under PAR there was a significant increase in leaf starch content of shoots pulsed with 5% glucose solutions when determined directly post-pulsing. The significant decrease in starch and glucose content of all tissues determined after 21 d storage was attributed to ongoing metabolic processes during storage. The bulk of the glucose taken up during pulsing was directed to the flowerhead, followed by the leaves and lastly stems. The fact that there was no significant difference in mean total glucose content between leaves and stems of shoots pulsed with 5% glucose solutions directly post-pulsing is thought to represent a temporary increase in stems during uptake and transport to both flowerhead and leaves. The significant suppression of leaf blackening

in 5% glucose pulsed shoots was attributed, in part, to the formation of starch in the leaves under PAR and the utilisation of this starch in conjunction with the increased glucose available, to support metabolic processes during storage.

Literature cited

Akamine, E.K., Goo, T. & Suehisa, R., 1979. Relationship between leaf darkening and chemical composition of leaves of *Protea*. Flor. Rev. 163, 62-63, 107-108.

Bieleski, R.L., Ripperda, J., Newman, J.P. & Reid, M.S., 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117, 124-127.

Bolles, E. & Hollenberg, C.P., 1997. The molecular genetics of hexose transport in yeasts. FEMS Microbiol. Rev. 21, 85-111.

Brink, J.A., 1987. The influence of the flowerhead on leaf browning of cut *Protea neriifolia*. Protea News 5, 11-12.

Brink, J.A. & de Swardt, G. H., 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R. Br.. Acta Hort. 185, 111-119.

Cowling, R.M. & Mitchell, D.T., 1981. Sugar composition, total nitrogen and accumulation of C_{14} assimilates in floral nectaries of *Protea* species. J. S. Afr. Bot. 47, 743-750.

Criley, R.A., Williamson, C. & Masutani, R., 1979. Vase life determinations for *Leucospermum cordifolium*. Flor. Rev. 163, 37-38, 80-82.

Dai, J-W., 1993. Postharvest leaf blackening in *Protea neriifolia* R. Br.. Ph.D. Diss., Univ. of Hawaii at Manoa, Honolulu.

De Swardt, G.H., 1979. Blackening of *Protea* leaves. SAPPEX Res. 13.

De Swardt, G.H., Pretorius, J. & Burger, L., 1987. The browning of foliage leaves in *Protea* - a review. Protea News 6, 4-9

Faragher, J.D., 1989. A review of research on postharvest physiology and horticulture of Australian native flowers. Acta Hort. 261, 249-256.

Ferreira, D.I., 1983. Prevention of browning of leaves of *Protea neriifolia* R.Br.. Acta Hort. 138, 273-276.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescences. Acta Hort. 185, 121-129.

Getz, H.P., Knauer, D. & Willenbrink, J., 1987. Transport of sugars across the plasma membrane of beetroot protoplasts. Planta 171, 185-196.

Gogarten, J.P. & Bentrup, F.W., 1989. Substrate specificity of the hexose carrier in the plasma membrane of *Chenopodium* suspension cells probes by transmembrane exchange diffusion. Planta 178, 52-60.

Goszczyñska, D.M. & Rudnicki, R.M., 1988. Storage of cut flowers. Hort. Rev. 10, 35-62.

Haasbroek, F.J., Rousseau, G.G. & de Villiers, J.F., 1973. Effect of gamma rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5, 33-42.

Halevy, A.H. & Mayak, S., 1974. Transport and conditioning of cut flowers. Acta Hort. 43, 291-306.

Halevy, A.H. & Mayak, S., 1979. Senescence and postharvest physiology of cut flowers. Part 1. Hort. Rev. 1, 204-236.

Halevy, A.H. & Mayak, S., 1981. Senescence and postharvest physiology of cut flowers. Part 2. Hort. Rev. 3, 59-143.

Ireland, J.P., Meynhardt, J.T. & Strauss, J.M., 1967. When proteas become sailors: treatment before shipping. Farming in South Africa 43, 33-35.

Jones, R.B., 1991a. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. Proc. Intl Protea Assoc. 6th Biennial Conf., Perth, Western Australia. pp 313-322.

Jones, R.B., 1991b. A pre-storage sucrose pulse protects *Leucadendron* var. 'Silvan Red' during long term dry storage at 1°C. Acta Hort. 298, 247-253.

Jones, R.B., McConchie, R., van Doorn, W.G. & Reid, M.S., 1995. Leaf blackening in cut *Protea* flowers. Hort Rev. 17, 173-201.

Lacey, S., 1982. Vase life of *Grevillea* Sandra Gordon. Proc. Production and Marketing of Australian Wildflowers for Export Conf., 1982. Univ. of Western Australia, Perth, Australia. pp 111-113.

Ligawa, J.K., Joyce, D.C. & Hetherington, S.E., 1997. Exogenously supplied sucrose improves the postharvest quality of *Grevillea* 'Sylvia' inflorescences. Aust. J. Exp. Agric. 37, 809-816.

Maynard, J.W. & Lucas, W.J., 1982. Sucrose and glucose uptake in *Beta vulgaris* leaf tissue. A case for a general (apoplastic) retrieval system. Plant Physiol. 70, 1436-1443.

McConchie, R. & Lang, N.S., 1993. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118, 355-361.

McConchie, R., Lang, N.S. & Gross, K.C., 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116, 1019-1024.

Mostert, D.P., Siegfried, W.F. & Louw, G.N., 1980. *Protea* nectar and satellite fauna in relation to the food requirements and pollinating role of the Cape sugarbird. S. Afr. J. Sci. 76, 409-412.

Mulder, P.W.A., 1977. Primêre meganismes betrokke by die bruinwording van loofblare in *Protea neriifolia*. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.

Nowak, J, Rudnicki, R.M. & Duncan, A.A., 1990. Postharvest handling and storage of cut flowers, florist greens, and potted plants. Timber Press, Portland, Oregon.

Özcan, S. & Johnston, M., 1995. Three different regulatory mechanisms enable yeast hexose transporter (HXT) genes to be induced by different levels of glucose. Mol. Cell. Biol. 15, 1564-1572.

Parvin, P.E., 1978. The influence of preservative solutions on bud opening and vase life of selected proteas. Proc. First Ann. Orn. Sem., Univ. Hawaii CES Misc. Publ. 173, 29-33.

Paull, R.E. & Dai, J-W, 1990. *Protea* postharvest blackleaf: a problem in search of a solution. Acta Hort. 264, 93-101.

Paull, R., Goo, T., Criley, R.A. & Parvin, P.E., 1980. Leaf blackening in cut *Protea eximia*: importance of water relations. Acta Hort. 113, 159-166.

Perez, A.G., Olias, R., Espada, J., Olias, J.M. & Sanz, C., 1997. Rapid determination of sugars, non-volatile acids and ascorbic acid in strawberry and other fruits. J. Agric. Food Chem. 45, 3545-3549.

Perold, G.W., 1993. Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. S. Afr. J. Sci. 89, 90-93.

Reid, M.S., van Doorn, W. & Newman, J.P., 1989. Leaf blackening in proteas. Acta Hort. 261, 81-84.

Santarius, K.A., 1973. The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. Planta 113, 105-114.

Statistical Analysis Systems Institute, 1996. SAS® User's Guide, Version 6.12, Cary, NC.

Tubbe, A. & Buckhout, T.J., 1992. *In vitro* analysis of the H^+-hexose symporter on the plasma membrane of sugarbeets (*Beta vulgaris* L.). Plant Physiol. 99, 945-951.

Van Wyk, B-E & Nicholson, S.W., 1995. Xylose is a major sugar in *Protea* and *Faurea*. S. Afr. J. Sci. 91, 151-152.

Vuthapanich, S., Simons, S. & Turnball, L.V., 1994. Effects of harvest maturity and postharvest treatments on vase life of *Grevillea* cv. 'Majestic' inflorescences. Proc. Australian Postharvest Conf. 1993. Univ. of Queensland, Gatton College, Gatton. pp 45-51.

Wiens, D., Rourke, J.P., Casper, B.B., Rickart, E.A., LaPine, T.R., Peterson, C.J. & Channing, A., 1983. Nonflying mammal pollination of southern African proteas: a noncoevolved system. Ann. Missouri Bot. Gard. 70, 1-31.

Tables

Table 1.

Effect of sucrose holding and pulsing solutions on vase life of 'Sylvia' protea cut flowers.

Treatment	Vase life (days)
Holding solution	
Control (distilled water)	6[a]
1% sucrose	7[a]
2% sucrose	3[b]
5% sucrose	2[b]
Pulsing solution	
Control (distilled water)	6[a]
10% sucrose	6[a]
20% sucrose	8[a]

Means (n=10) followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 2.

Concentration of sucrose, glucose and fructose ($mg \cdot g^{-1}$ dry weight) in 'Sylvia' leaves and flowerhead at harvest, after 3 d storage at 0°C in darkness, and a subsequent period of 2 and 6 d at 18±1°C in natural light.

Treatment	Sucrose ($mg \cdot g^{-1}$)	Glucose ($mg \cdot g^{-1}$)	Fructose ($mg \cdot g^{-1}$)
Leaves			
Harvest	0.856[a]	3.449[a]	11.766[a]
3 d at 0°C	0.527[b]	2.563[b]	11.928[a]
3 d at 0°C + 2 d at 18°C	0.856[a]	0.287[c]	8.892[b]
3 d at 0°C + 6 d at 18°C	0.072[c]	0.150[c]	9.401[b]
Flowerhead			
Harvest	0.503[c]	3.156[a]	7.868[a]
3 d at 0°C	0.323[c]	2.892[a]	8.509[a]
3 d at 0°C + 2 d at 18°C	2.168[a]	2.467[b]	7.353[a]
3 d at 0°C + 6 d at 18°C	1.383[b]	2.120[c]	8.042[a]

Mean (n=6) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 3.

Effect of glucose pulsing solutions on vase life of 'Sylvia' protea cut flowers. Shoots were pulsed at 18±1°C for 24 h before being placed in individual vases containing tap water and vase life assessed in a controlled temperature room (18±1°C) subject to natural light.

Treatment	Vase life (days)
Control (water)	5^{d}
1% glucose	5^{d}
2% glucose	6^{d}
3% glucose	8^{c}
4% glucose	9^{c}
5% glucose	14^{a}
6% glucose	14^{a}
8% glucose	11^{b}
10% glucose	12^{b}

Means (n=10) followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 4.

Effect of harvest time, pulsing temperature and supplemental photosynthetic lighting (140 $\mu mol \cdot m^{-2} s^{-1}$) on time to take up 10 mL of 5% glucose solution by 'Sylvia' protea cut flowers.

	Uptake time (h)	
Pulsing treatment	Morning harvest	Afternoon harvest
Dark		
20°C	21.9[a]	2.5[a]
25°C	17.9[c]	2.0[b]
30°C	20.0[b]	1.9[bc]
Light		
20°C	4.5[d]	2.0[b]
25°C	2.1[e]	1.5[c]
30°C	2.5[e]	1.0[d]

Means (n=10) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 5.

Effect of harvest temperature, pulsing temperature and supplemental photosynthetic lighting (140 $\mu mol \cdot m^{-2} s^{-1}$) on 'Sylvia' protea cut flower vase life.

	Vase life (days)	
Pulse treatment	Morning harvest	Afternoon harvest
Dark		
20°C	7.2[a]	10.0[b]
25°C	8.0[b]	9.2[a]
30°C	7.7[ab]	9.2[a]
Light		
20°C	9.7[c]	10.0[b]
25°C	9.7[c]	9.9[b]
30°C	9.6[c]	9.7[ab]

Means (n=10) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 6.

Percentage mass loss by 'Sylvia' protea cut flowers pulsed with either 0 (control) or 5% glucose solution (10 mL, 25°C, 140 $\mu mol \cdot m^{-2} s^{-1}$) after 21 d storage (1°C, 95% RH) inside PE lined and enclosed cartons or unlined, unenclosed (control) cartons.

	% mass loss	
Treatment	Unlined + unenclosed carton	Lined + enclosed carton
0% glucose	5.94[a]	1.75[b]
5% glucose pulse	3.50[a]	0.89[b]

Means (n=20) within columns followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Figures

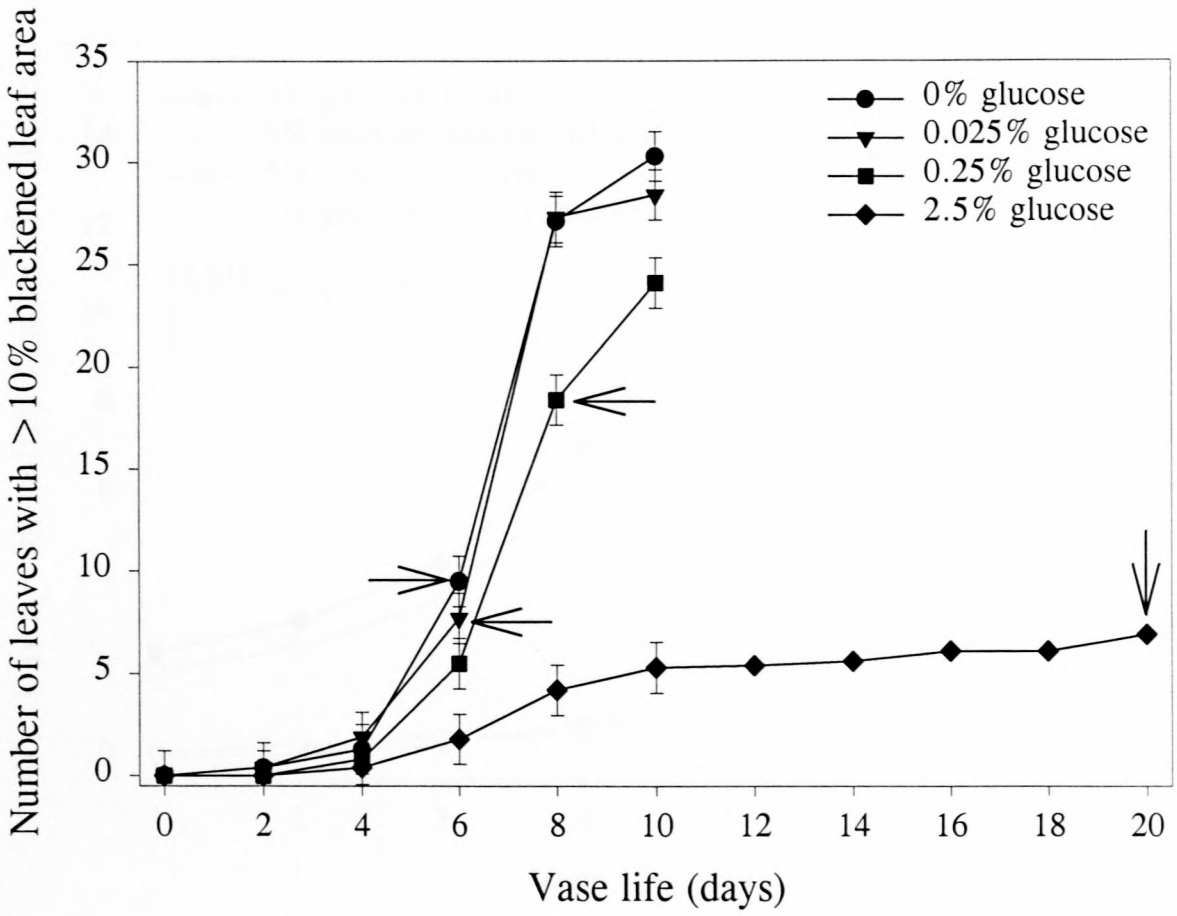
Fig. 1. Number of leaves with at least 10% blackened leaf area per shoot placed in glucose holding solutions during vase life evaluation at 18°C. After 10 days, vase life assessment was terminated due to excessive leaf blackening for all but those shoots in 2.5% glucose. Vertical bars represent S.E. (n=10). Arrows indicate end of vase life.

Fig. 2. Appearance of 'Sylvia' protea cut flowers subjected to 0 and 2.5% glucose holding solutions after 10 d vase life assessment.

Fig. 3. Number of leaves with ≥10% blackened leaf area per shoot during vase life assessment (8 d, 18°C). Shoots were pulsed with either 0 or 5% glucose solutions prior to being packed into either PE lined or unlined (control) cartons. Shoots were vacuum cooled prior to storage for 21 d (1°C, 95% RH). Lined cartons were sealed inside PE bags. Data represented are the means of 10 shoots.

Fig. 4. Total starch content (mg) of flowerhead, leaves and stem per shoot determined at harvest, directly after pulse treatment (0 and 5% glucose, 10 mL, 25°C, 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and after 21 d storage (1°C, 95% RH). Data represented are the means of 12 shoots. Different letters above columns indicate significant differences within plant part at $LSD_{(P=0.05)}$ level.

Fig. 5. Total glucose content (mg) of flowerhead, leaves and stem per shoot determined at harvest, directly post-pulse treatment (0 and 5% glucose, 10 mL, 25°C, 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and after 21 d storage (1°C, 95% RH). Data represented are the means of 12 shoots. Different letters above columns indicate significant differences within plant part at $LSD_{(P=0.05)}$ level.



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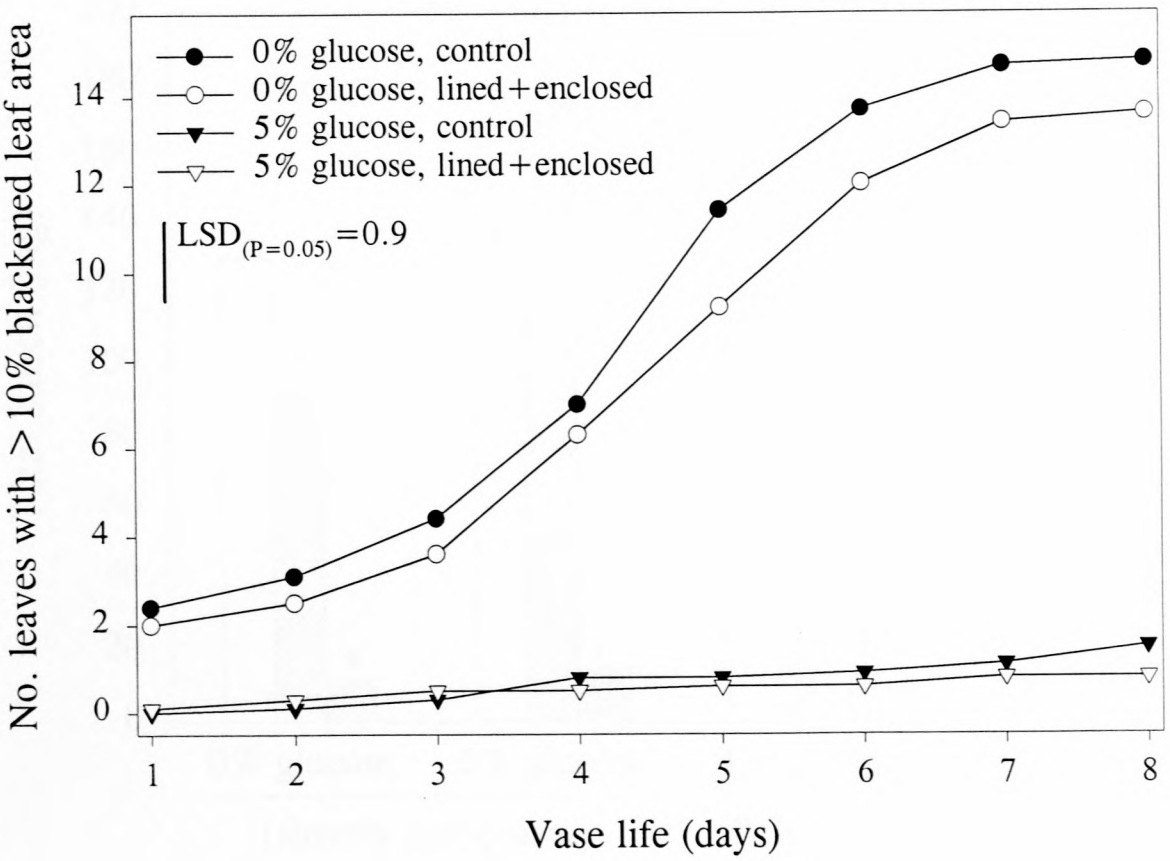

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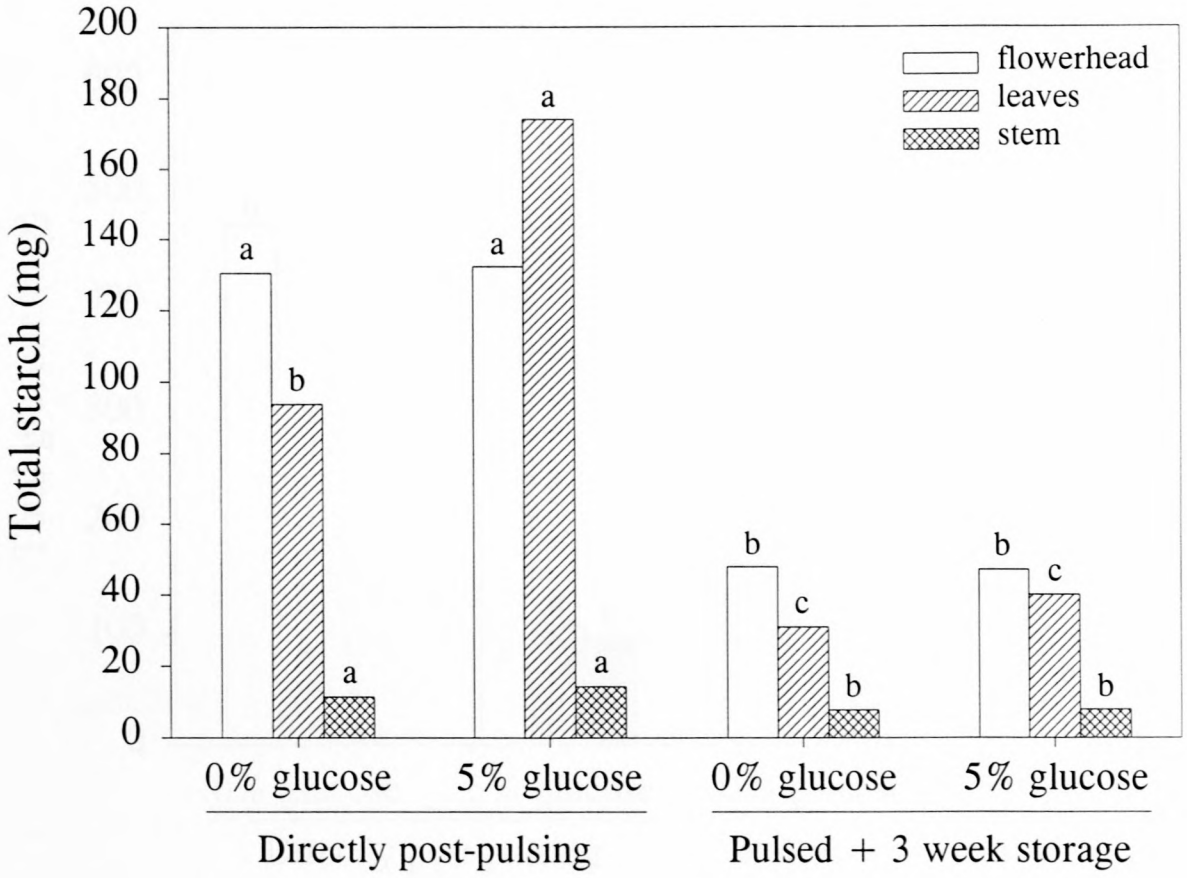

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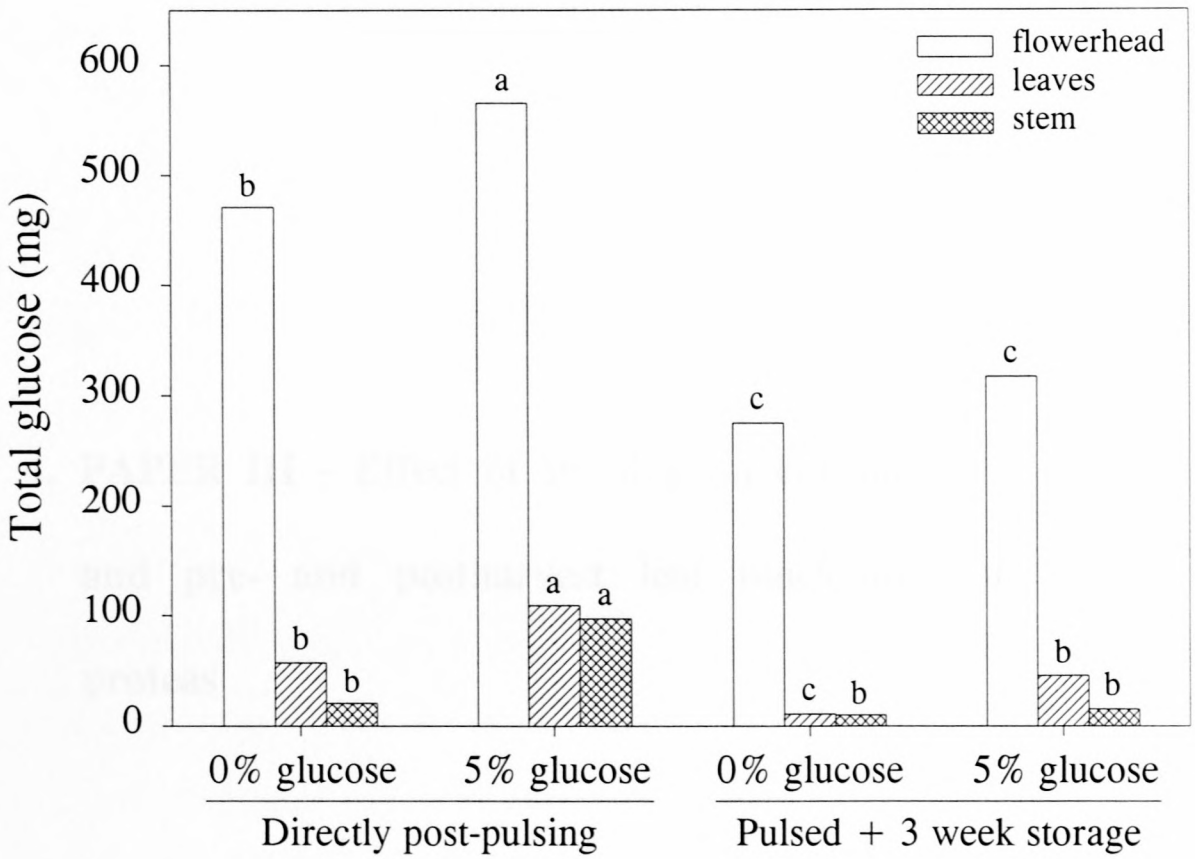


Fig. 5.

Total glucose content (mg) of flowerhead, leaves and stem per shoot determined at harvest, directly post-pulse treatment (0 and 5% glucose, 10 mL, 25°C, 140 μmol·m^{-2}·s^{-1}) and after 21 d storage (1°C, 95% RH). Data represented are the means of 12 shoots. Different letters above columns indicate significant differences within plant part at $LSD_{(P=0.05)}$ level.

4. PAPER III - Effect of shading on carbohydrate content and pre- and postharvest leaf blackening of 'Sylvia' proteas

Effect of shading on carbohydrate content and pre- and postharvest leaf blackening of 'Sylvia' proteas

Leaf blackening has been defined as regions of dark brown to black discolouration appearing in various regions of the leaf (de Swardt, 1979). Preharvest leaf blackening occurs in a number of *Protea* species and hybrids formed from them, for example *P. eximia*, *P.* cv. Cardinal (*P. eximia* × *P. susannae*), *P.* cv. Sylvia (*P. eximia* × *P. susannae*), *P. repens* and *P. neriifolia* (pers. observations). Mechanical damage, insect or fungal attack, water stress (Starke, 1979; Forsberg, 1993) or excessive heat (Ferreira, 1986) may result in preharvest leaf blackening. This is usually associated with physical damage, usually at the center of the affected region, or, in the case of heat stress, is apparent as a light brown colouration across the leaf.

The incidence of leaf blackening in 'Sylvia' proteas grown in South Africa increases during the months of July-September, affecting the crop exported during this period. The increase in postharvest leaf blackening corresponds to reduced daylength and light levels of the South African winter whilst preharvest leaf blackening may also occur at this time. Hettasch (1999) found total sugar and starch concentration decreased in *Protea* cvs Sylvia and Cardinal through winter, whilst sugar levels of *Leucospermum* cv. Red Sunset (*L. cordifolium* × *L. lineare*) leaves decreased under shading (Napier, 1985). Postharvest leaf blackening has been correlated with decreased carbohydrate content in several *Protea* cut flowers (McConchie *et al*, 1991;

Bieleski *et al.*, 1992; McConchie & Lang, 1993) and it is thought that a similar process might be involved in preharvest leaf blackening of 'Sylvia' proteas.

Preharvest leaf blackening of 'Sylvia' proteas flowering shoots significantly reduces quality and marketability of the cut flowers. At best, preharvest leaf blackened shoots require significant postharvest remedial action, involving selective leaf removal and leaf trimming, which increases labour input and reduces profitability (pers. observations). Our hypothesis was that accentuating the winter conditions of lower light levels, by preharvest shading, would result in increased pre- and postharvest leaf blackening of 'Sylvia' flowering shoots.

Materials and Methods

PLANT MATERIAL. Five-year old 'Sylvia' plants in a commercial plantation located in the Stellenbosch district (33°55'S; 18°50'E), South Africa were used. The climate is Mediterranean, with cool, wet winters and hot, dry summers. Annual rainfall is 600-700 mm. Plants were spaced 1 x 4 m, clean cultivated, fertilised, irrigated and sprayed according to commercial practices.

Shoot elongation in *Protea* occurs by successive growth flushes, usually named after the season in which they are formed. In spring a vigorous growth flush occurs subsequent to winter dormancy. This is followed by either one or two growth flushes in summer and a less vigorous autumn flush (Malan & Le Roux, 1995). Shoots that formed a reproductive bud on shoots bearing four and five vegetative flushes were used (Fig. 1). Shoots were shaded with 50% shadecloth bags when reproductive bud

diameter was 16–18 mm (four flush shoot) or 12–15 mm (five flush shoot). Shading of four flush shoots was done on June 3rd, 1999, whilst that of five flush shoots was done on June 24th, 1999. Shoots were selected to be of similar stem length and leaf number with no visual signs of preharvest leaf blackening.

DRY MASS. Shaded four and five flush shoots described and unshaded shoots as control were used. Control shoots were tagged at the beginning of the trial to ensure similar initial characteristics. Individual shoots were selected from different plants. Shoots were harvested at 21 d intervals and the number of leaves and length of the subtending and older flushes were determined. Flowerhead basal diameter was measured using digital callipers at each sample date. The number of preharvest blackened leaves was determined prior to shoots being separated into flowerhead, leaf and stem components for carbohydrate analyses. Subtending flush leaves and subtending flush stems formed individual samples. Leaves from older flushes were combined to form one sample as were stems from older flushes. Samples were lyophilised and the dry mass determined before being milled to a fine powder.

CARBOHYDRATE ANALYSIS. Carbohydrate analyses of flowerhead starch and reducing sugars are reported in terms of total content (g) whilst that of subtending and older flush tissues are reported in terms of concentration ($mg \cdot g^{-1}$).

A 0.5 g sample of the dried tissue described was taken for carbohydrate analysis. Samples were extracted for 15 h in 1% acetic acid by shaking and then centrifuged (10 000 g_n, 12 min, 4°C). Supernatant was filtered and made up to 100 mL with 1% acetic acid. Thereafter, the pellet was dissolved in an acetate buffer (pH 4.8) and

gelatinised in a boiling steam bath for 2 h. The suspension was cooled to 60°C and the starch fraction hydrolysed to glucose with amyloglucosidase (EC 3.2.1.3) (Fluka Chemie, Buchs, Switzerland). Hydrolysis was performed in an incubator maintained at 55°C for 18 h. Analysis of reducing sugars and starch was done on a Sanplus Segmented Flow Analysis System (Method Number 551-965w/r issue 070798/MH and Number 356-001w/r issue 012998/MH97203066; Skalar, De Breda, The Netherlands). Five single shoot replicates per treatment were used.

VASE LIFE ASSESSMENT OF PREHARVEST SHADED SHOOTS. Extrapolation of flowerhead basal diameter data (Gerber, unpublished data) was done to enable shading of individual five flush flowering shoots with 50% shadecloth bags at 15, 4, 3, 2 and 1 week prior to harvest. Unshaded shoots served as control. As involucral bracts began to loosen shoots were cut into water and brought to the laboratory within 1 h. The number of blackened leaves on 20 shoots per shading treatment was determined. Ten single shoot replicates with non-blackened leaves were used for vase life assessment. Shoots were recut to 55 cm and the bottom 20 cm stripped of leaves. Vase life was assessed in a controlled temperature room (18 ± 1°C) subject to natural light.

STATISTICAL ANALYSIS. Standard analysis of variance was performed using the SAS® Enterprise Guide® program (Statistical Analysis Systems Institute, 1999). Students t-LSD were calculated at a 5% significance level to compare treatment means.

Results

DRY MASS. Mean number of leaves and length of the different flushes of four and five flush flowering shoots are given in Table 1. The number of leaves and length of the combined older flushes of five flush shoots was significantly greater than that of the four flush shoots. Shading treatment appeared to have no significant effect on flowerhead development as determined by basal diameter (Fig. 2). Anthesis of four and five flush shoots was reached in 12 and 15 weeks respectively. Preharvest leaf blackening did not occur on flowering shoots shaded for 12 or 15 weeks (data not presented). Dry mass increased significantly and continuously in the developing flowerhead on both four and five flush shoots (Figs 3 & 4 respectively). There were small yet significant increases in dry mass of stems of subtending and older flushes of four and five flush shoots, and in the subtending flush leaves of five flush shoots.

CARBOHYDRATE ANALYSES.

A. STARCH.

FOUR FLUSH SHOOTS. Total starch content of the flowerhead and starch concentration of the subtending and older flush tissues are shown in Fig. 5. Total starch content of the flowerhead increased significantly with time. However, no significant difference between treatments was found at each sampling. Subtending flush leaf starch concentration increased with time, and was significantly lower in shaded subtending flush leaves from six weeks post-shading onward. Starch concentration of older flush leaves was significantly lower in shaded shoots at 6 and 12

weeks post-shading. Starch concentration of stems from subtending and older flushes was significantly lower in shaded tissue after 12 weeks post-shading.

FIVE FLUSH SHOOTS. Total starch content of the flowerhead and starch concentration of subtending and older flush tissues is shown in Fig. 6. Total starch content increased significantly in the developing flowerhead and was unaffected by shade treatment. Starch concentration was significantly lower in shaded subtending flush tissues from onset of sample collection. Starch concentration of shaded older flush leaves was significantly lower than unshaded control from six weeks post-shading onward. Starch concentration of shaded older flush stems was significantly lower three weeks post-shading.

B. REDUCING SUGARS.

FOUR FLUSH SHOOTS. Total reducing sugar content of the flowerhead and reducing sugar concentration of subtending and older flush tissues are shown in Fig.7. Total reducing sugar content of the developing flowerhead increased significantly with time irrespective of shade treatment. Reducing sugar concentration of subtending flush leaves was significantly greater under shade at three weeks post-shading. In older flush leaves starch concentration was significantly greater in shaded shoots after 12 weeks post-shading. No significant difference in reducing sugar concentration was found in either subtending or older flush stems until 12 weeks post-shading when a higher concentration was found in unshaded control shoots.

FIVE FLUSH SHOOTS. Total reducing sugar content of the flowerhead and reducing sugar concentration of subtending and older flush tissues are shown in Fig. 8. Total reducing sugar content of the developing flowerhead increased significantly with time and was unaffected by shade treatment. Reducing sugar concentration of shaded subtending flush leaves was significantly lower 12 weeks post-shading onward, whilst that of shaded subtending flush stems was significantly lower at 3, 9 and 15 weeks post-shading. Reducing sugar concentration decreased with time in shaded subtending flush leaves and subtending flush stems irrespective of shading treatment. Reducing sugar concentration was significantly lower in shaded older flush stems at three weeks post-shading only. No significant difference in older flush leaf reducing sugar concentration between shade treatments was found.

VASE LIFE ASSESSMENT OF PREHARVEST SHADED SHOOTS. Preharvest leaf blackening on shoots shaded four weeks preharvest was significantly higher than other shade treatments (Table 2). Preharvest leaf blackening had not occurred on unshaded control shoots at harvest. No significant difference in the number of leaves with at least 10% blackened leaf area was found between shading treatments. Postharvest leaf blackening first appeared after 2 d at 18°C and by 6 d was sufficiently severe to terminate usable vase life (Fig. 9).

Discussion

Shoot elongation in *Protea* occurs by successive growth flushes, usually named after the season in which they are formed. In spring a vigorous growth flush occurs subsequent to winter dormancy. This is followed by either one or two growth flushes in summer and a less vigorous autumn flush (Malan & Le Roux, 1995). Anthesis was reached later in five flush shoots due to later flowerhead initiation. Shading of five flush shoots was also done on shoots with a smaller reproductive bud, which may explain the longer time to reach anthesis. Dry mass and number of leaves of the older flush of five flush shoots was significantly greater than that of four flush shoots due to the formation of an additional growth flush. Hettasch (1999) reported on dry mass accumulation in vegetative four flush shoots of one-year old 'Sylvia' plants. The values reported in the current research are lower and are thought to reflect differences in plant vigour, time of year in which shoot formation occurred and the use of flowering instead of vegetative shoots. The small increase in stem dry mass of both four and five flush shoots is attributed to secondary thickening. The increase in dry mass of subtending leaves of five flush shoots was attributed to the hardening of leaves, subsequent to expansion, through the formation of a waxy cuticle (Gerber, 2000).

Starch and reducing sugar content of the developing flowerhead was unaffected by shading treatment in both four and five flush shoots and this was attributed to flowerhead sink strength. Starch concentration of stem and leaf tissue was affected to a greater extent than reducing sugar concentration in both four and five flush shoots.

Shaded four flush shoots had a significantly lower starch concentration in subtending flush leaves, which may be due to photosynthates being directed to the strong developing flowerhead sink. The significantly lower starch concentration, and concurrently greater reducing sugar concentration, of shaded 4 flush older flush leaves was attributed to mobilisation of starch reserves for translocation to developing flowerhead.

Shading of five flush shoots resulted in a significantly lower starch concentration in subtending flush tissue and older flush leaves. This was possibly due to suppression of photosynthesis and translocation of photosynthates to the developing flowerhead. Reducing sugar concentration of subtending and older flush tissue of five flush shoots decreased with time and may reflect movement to developing flowerhead.

We had expected that shading, which accentuated winter conditions, would result in increased preharvest leaf blackening and earlier onset of postharvest leaf blackening. Shading four and three weeks prior to harvest coincided with a period of a significant increase in flowerhead dry mass. It is possible that shading at this point, concurrent with an increased carbohydrate demand by the developing flowerhead, caused a temporary limitation in photosynthate production and supply, resulting in the appearance of preharvest leaf blackening. Some of the carbohydrate in leaves of *Protea* species susceptible to leaf blackening is bound with phenolics to form unstable *O*-glycoside esters (Perold *et al.*, 1973a,b; Perold *et al.*, 1979; Perold & Carlton, 1989; Perold, 1993). Perold (1993) hypothesised that the demand for carbohydrates for the developing flowerhead may stimulate or cause the cleavage of these compounds

allowing the sugar(s) to be translocated to the flowerhead, and releasing a reactive phenolic moiety which can undergo non-enzymatic oxidation resulting in leaf blackening. It is possible that phenolic-bound carbohydrate was utilised at this point to overcome a carbohydrate supply deficit and, in accordance with Perold's (1993) hypothesis, that this resulted in preharvest leaf blackening.

That no preharvest leaf blackening was found in shoots shaded 15 weeks prior to harvest may reflect an adaptation to reduced light levels, presumably by an increase in photosynthetic efficiency. Shading at two and one week prior to harvest corresponded to a period where flowerhead dry mass accumulation was much lower. It is possible that carbohydrate demands were lower at this time and, although shading may have suppressed photosynthate production, carbohydrate demands of the developing flowerhead were met.

The fact that shading did not enhance leaf blackening postharvest may be due to the low levels of carbohydrate found in winter and although shading did result in a decrease in carbohydrate content, shoots were already susceptible to leaf blackening. Greenfield *et al.* (1995) reported low carbohydrate levels in the two-year old bearers of 'Carnival' proteas whilst Hettasch (1999) reported low levels of carbohydrate in vegetative shoots of both 'Carnival' and 'Sylvia' proteas. In 'Lady Di' proteas the carbohydrate content of mature tissue did not change significantly, indicating that current photosynthates were of greater importance during flowerhead development (Gerber *et al.*, 2001).

Although preharvest leaf blackening was not found on unshaded control shoots used in this trial, unshaded flowering shoots of 'Sylvia' proteas can be affected by preharvest leaf blackening (pers. observations). This preharvest leaf blackening occurs predominantly post-winter when plants are undergoing formation of a vigorous vegetative spring flush. It is possible that the mature flowering shoot unit serves as a source of carbohydrate for developing vegetative shoots, which, if too many in number, could result in a demand that cannot be met by flowering shoot photosynthesis. In this case cleavage of the glycoside esters described is possible and with resultant leaf blackening. A second possibility may involve increased levels of plant growth regulators, e.g. gibberellins and cytokinins, during spring flush development. These plant growth regulators may have an adverse effect on flowering shoots by directing photosynthates away from the developing flowerhead to the developing shoots resulting in a carbohydrate deficit as outlined above. Furthermore, gibberellins are known to promote cell growth by the loosening of cell walls (Reid & Howell, 1995) and increase hydrolytic enzymes production (Jacobsen *et al.*, 1995) and it is possible that these processes enhance access of hydrolytic enzymes to substrate promoting leaf blackening.

Further work investigating the interaction of light, vegetative shoot growth and plant growth regulators on preharvest leaf blackening is required.

Literature Cited

Bieleski, R.L., Ripperda, J., Newman, J.P. & Reid, M.S., 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117, 124-127.

De Swardt, G.H., 1979. Blackening of *Protea* leaves. SAPPEX Res. Paper 13.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescences. Acta Hort. 185, 121-129.

Forsberg, L.I., 1993. Protea diseases and their control. Queensland Dept Primary Industries, Brisbane, Queensland.

Gerber, A.I., 2000. Flower initiation and development in selected cultivars of the genus *Protea*. Ph.D. Diss., Univ. of Stellenbosch, Stellenbosch, South Africa.

Gerber, A.I., Theron, K.I. & Jacobs, G., 2001. The role of leaves and carbohydrates in flowering of *Protea* cv. Lady Di. HortScience 36, 905-908.

Greenfield, E.J., Theron, K.I. & Jacobs, G., 1995. Seasonal changes in carbohydrate and nitrogen levels in the bark and wood of pruned and unpruned plants of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 5, 25-28.

Hettasch, H.B., 1999. Studies of the vegetative development of *Protea* cv. 'Sylvia' and 'Cardinal'. M.Sc. Thesis, Univ. of Stellenbosch, Stellenbosch, South Africa.

Jacobsen, J.V., Gubler, F. & Chandler, P.M., 1995. Gibberellin action in germinated cereal grains. In: P.J. Davies (ed.), Plant hormones. 2nd Ed.. Kluwer Academic Publishers, Dordrecht. pp 246-271.

Malan, D.G. & Le Roux, R.D., 1995. Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of *Protea*. Acta Hort 387, 91-97.

McConchie, R., Lang, N.S. & Gross, K.C., 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116, 1019-1024.

McConchie, R. & Lang, N.S., 1993. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118, 355-361.

Napier, D.R., 1985. Initiation, growth and development of *Leucospermum* cv. Red Sunset inflorescences. M.Sc. Thesis, Univ. of Stellenbosch, Stellenbosch, South Africa.

Perold, G.W., 1993. Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. S. Afr. J. Sci. 89, 90-93.

Perold, G.W., Beylis, P. & Howard, A.S., 1973a. Metabolites of the Proteaceae. Part VII. Lacticolorin, a phenolic glucoside ester, and other metabolites of *Protea lacticolor* Salisb.. J. Chem. Soc. Perkin Trans I 1973, 638-642.

Perold, G.W., Beylis, P. & Howard, A.S., 1973b. Metabolites of Proteaceae. Part VIII. The occurrence of (+)-D-allose in nature: rubropilosin and pilorubrosin from *Protea rubropilosa* Beard. J. Chem. Soc. Perkin Trans I 1973, 643-649.

Perold, G.W., Rosenberg, M.E.K., Howard, A.S. & Huddle, P.A., 1979. Metabolites of Proteaceae. Part 9. Eximin (6-*O*-benzoylarbutin) and the synthesis of aryl glucoside ester. J. Chem. Soc. Perkin Trans I 1979, 239-243.

Perold, G.W. & Carlton, L., 1989. Neriifolin, an ester glucoside of 1,2,4-trihydroxybenzene. J. Chem. Soc. Perkin Trans I 1989, 1215-1217.

Reid, J.B. & Howell, S.H., 1995. Hormone mutants and plant development. In: P.J. Davies (ed.), Plant hormones. 2nd Ed.. Kluwer Academic Publishers, Dordrecht. pp 448-485.

Starke, L.C., 1979. Common *Protea* pests. Farming in South Africa: Flowers and Ornamental Shrubs Publ. B.11.

Statistical Analysis Systems Institute, 1999. SAS® Enterprise Guide® User's guide, Version 1.2. Cary, NC.

Tables

Table 1.

Mean leaf number and stem length of subtending and older flushes of shoots comprising four vegetative flushes (mean of 50 samples ±1SE) and five vegetative flushes (60 samples ±1SE).

	Subtending flush		Older flushes	
Flowering Shoot	Leaves	Stem length (cm)	Leaves	Stem length (cm)
Four flush	28.0±0.8	19.2±0.9	53.4±1.6	39.2±1.3
Five flush	32.2±1.2	18.4±0.6	80.9±1.9	56.8±1.7

Table 2.

Effect of time of shading on preharvest leaf blackening of *Protea* cv. Sylvia flowering shoots. Data represented are the means of 20 shoots per shading treatment.

Time of preharvest shading	Number of blackened leaves
No shade (control)	0^{c}
1 week	0^{c}
2 weeks	0^{c}
3 weeks	0.9^{b}
4 weeks	2.3^{a}
15 weeks	0^{c}

Means followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Figures

Fig. 1. Terminology to describe *Protea* cut flowers. The terminal flush upon which the flowerhead is borne is known as the subtending flush. The older flushes of four flush flowering shoots comprised three vegetative flushes whilst that of five flush flowering shoots comprised four vegetative flushes.

Fig. 2. Effect of shading (0 and 50%) on developing flowerhead basal diameter of 'Sylvia' proteas borne on four and five flush shoots. Anthesis was reached in 12 and 15 weeks by four and five flush flowering shoots respectively. Data represented are the averages of 5 shoots per treatment.

Fig. 3. Effect of shading (0 and 50%) on dry mass of four flush flowering shoots during flowerhead development. Data represented are the average of 10 shoots.

Fig. 4. Effect of shading (0 and 50%) on dry mass of five flush flowering shoots during flowerhead development. Data represented are the average of 10 shoots.

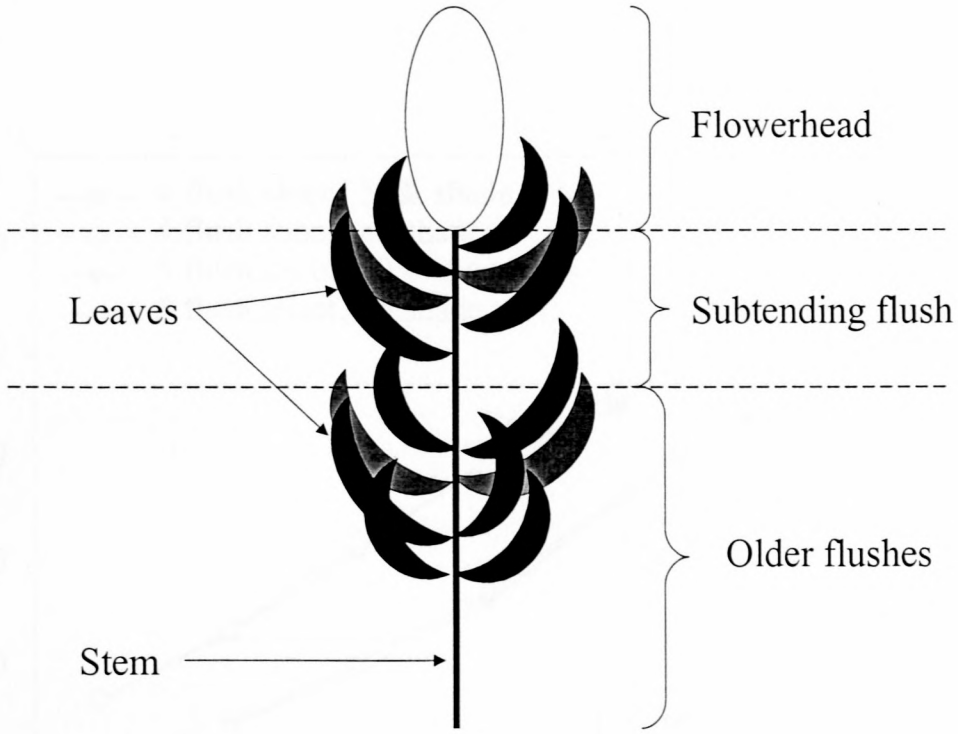
Fig. 5. Effect of shading (0 and 50%) on starch content of four flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ over time

Fig. 6. Effect of shading (0 and 50%) on starch content of five flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ over time

Fig. 7. Effect of shading (0 and 50%) on reducing sugar content of four flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ over time

Fig. 8. Effect of shading (0 and 50%) on reducing sugar content of five flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ over time

Fig. 9. Effect of preharvest shading on postharvest leaf blackening of 'Sylvia' protea cut flowers. Data represented are the averages of 10 shoots.



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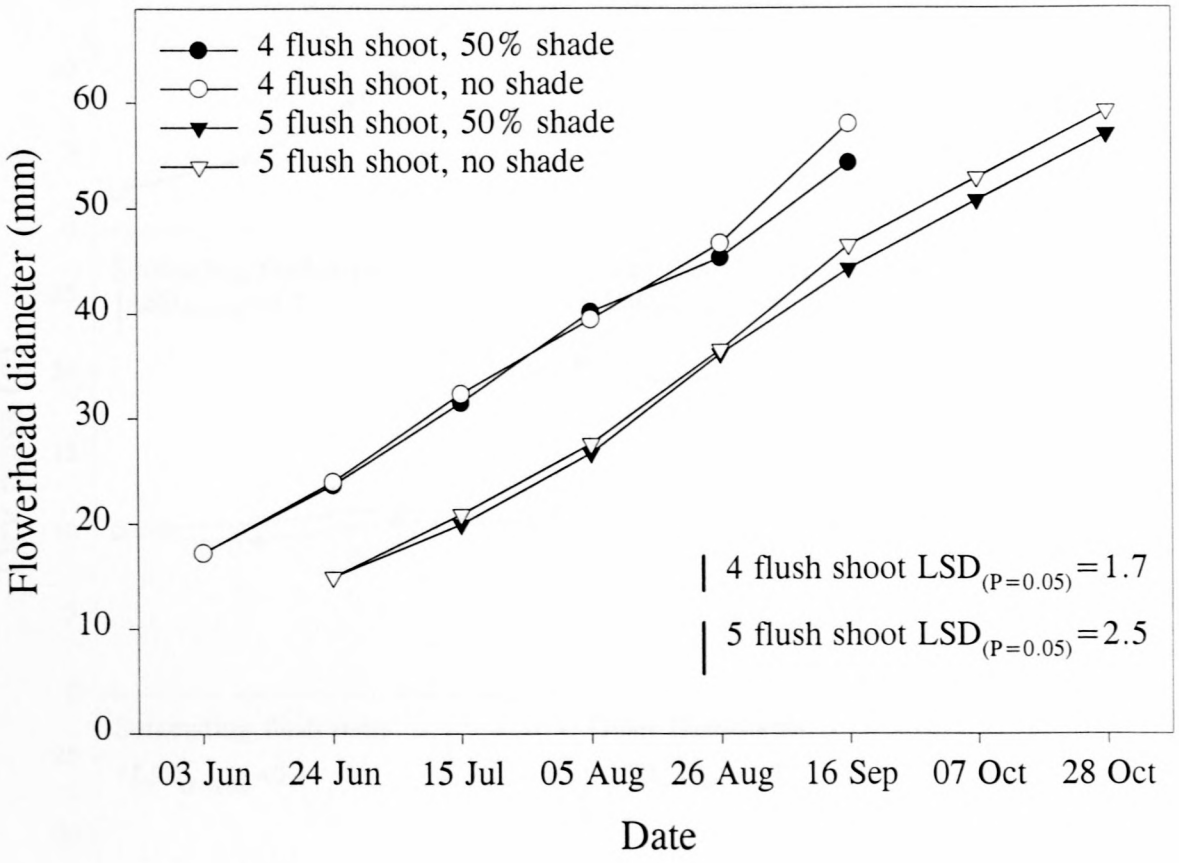

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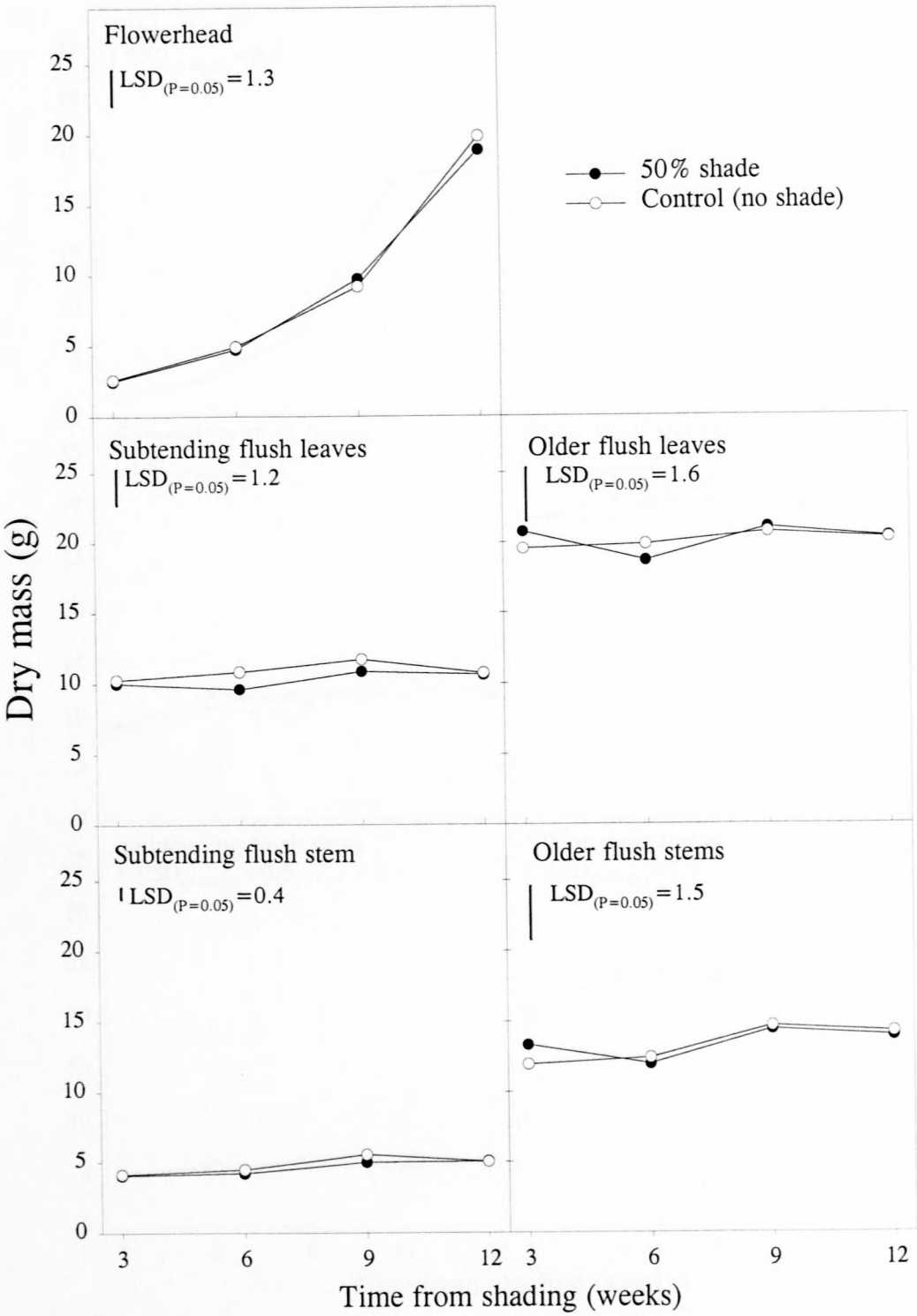


Fig. 3.
Effect of shading (0 and 50%) on dry mass of four flush flowering shoots during flowerhead development. Data represented are the average of 10 shoots. Vertical bars represent $LSD_{(P=0.05)}$ values over time.

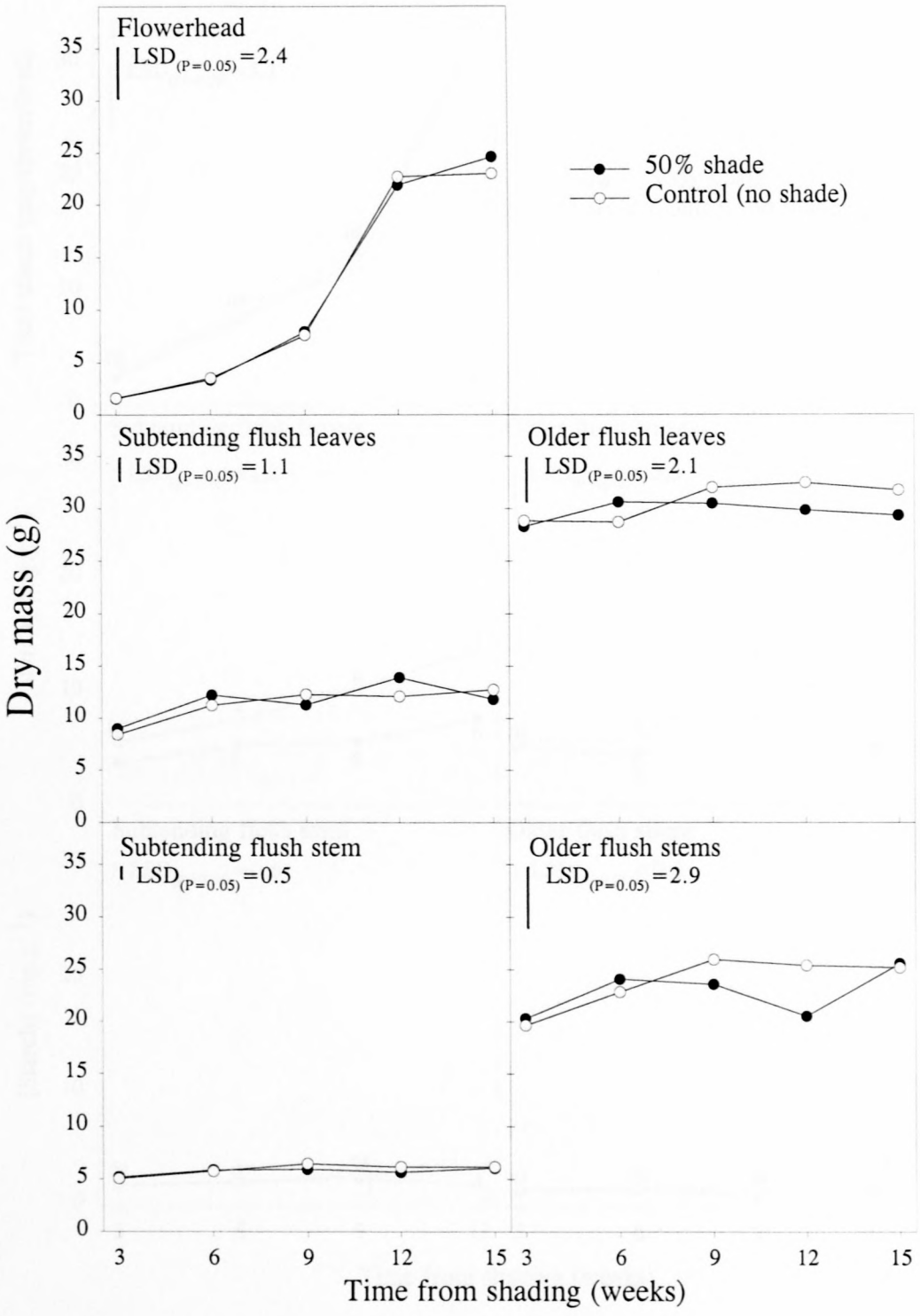


Fig. 4.
Effect of shading (0 and 50%) on dry mass of five flush flowering shoots during flowerhead development. Data represented are the average of 10 shoots. Vertical bars represent $LSD_{(P=0.05)}$ values over time.

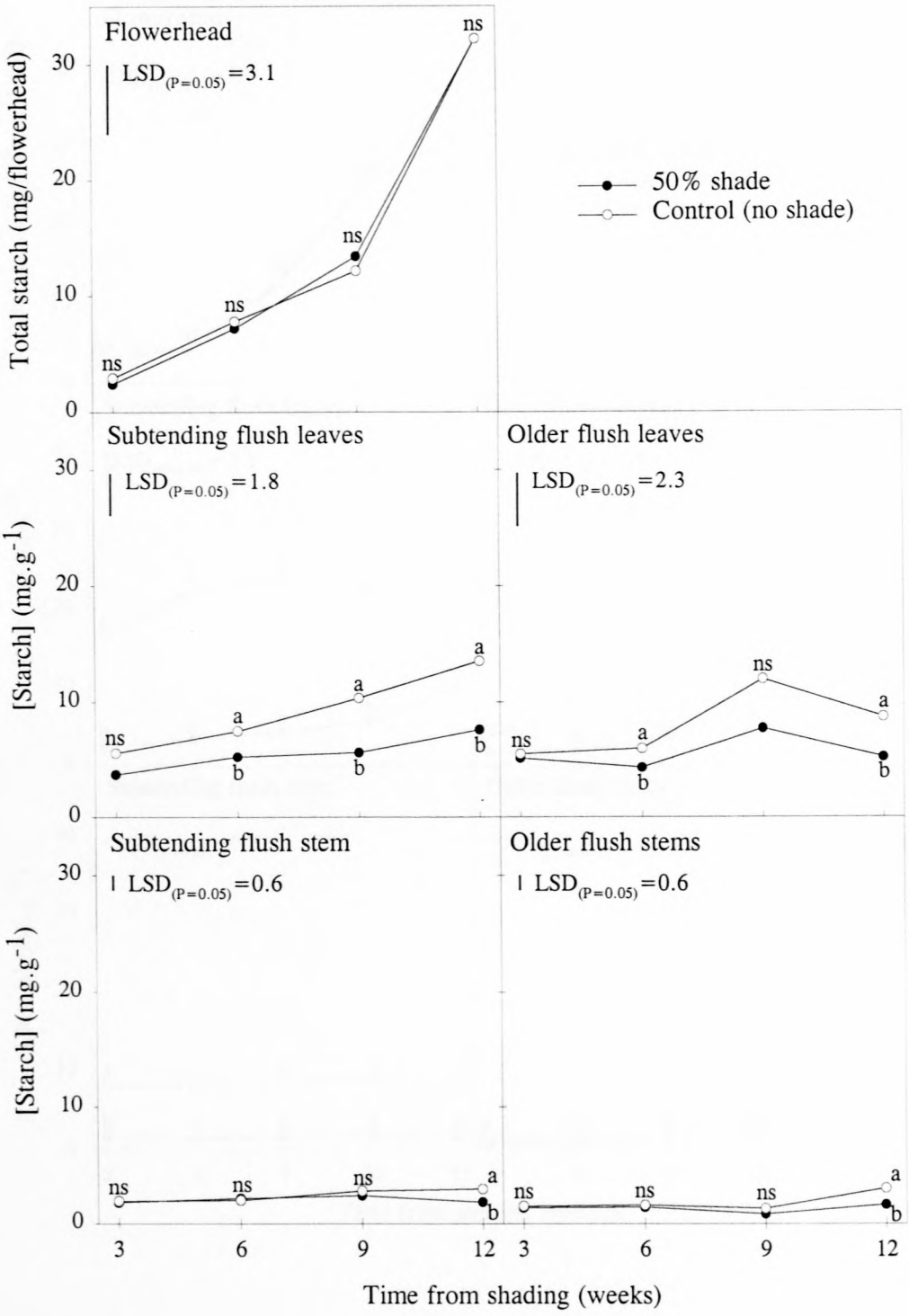


Fig. 5.
Effect of shading (0 and 50%) on starch content of four flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ values over time.

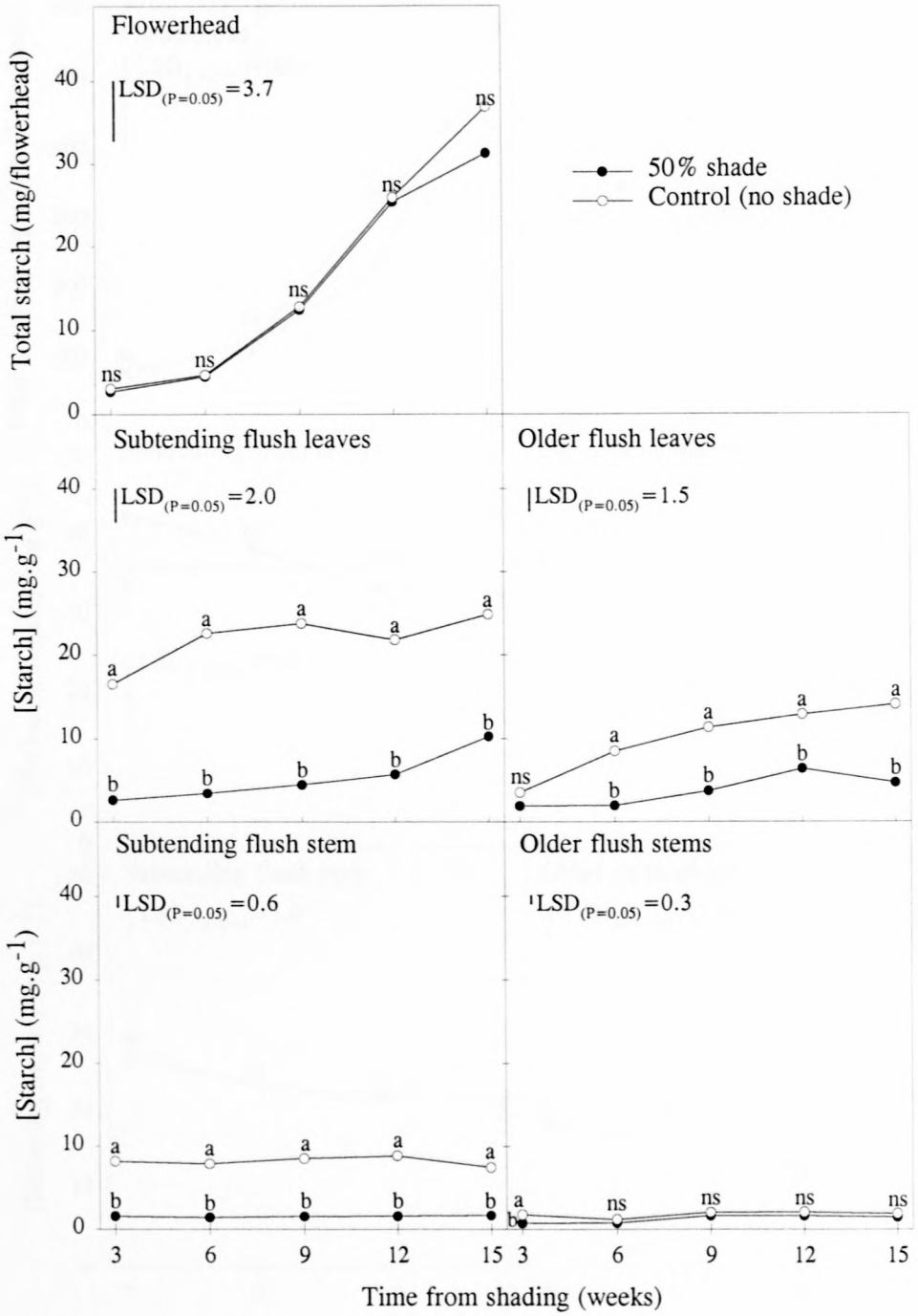


Fig. 6.
Effect of shading (0 and 50%) on starch content of five flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ values over time.

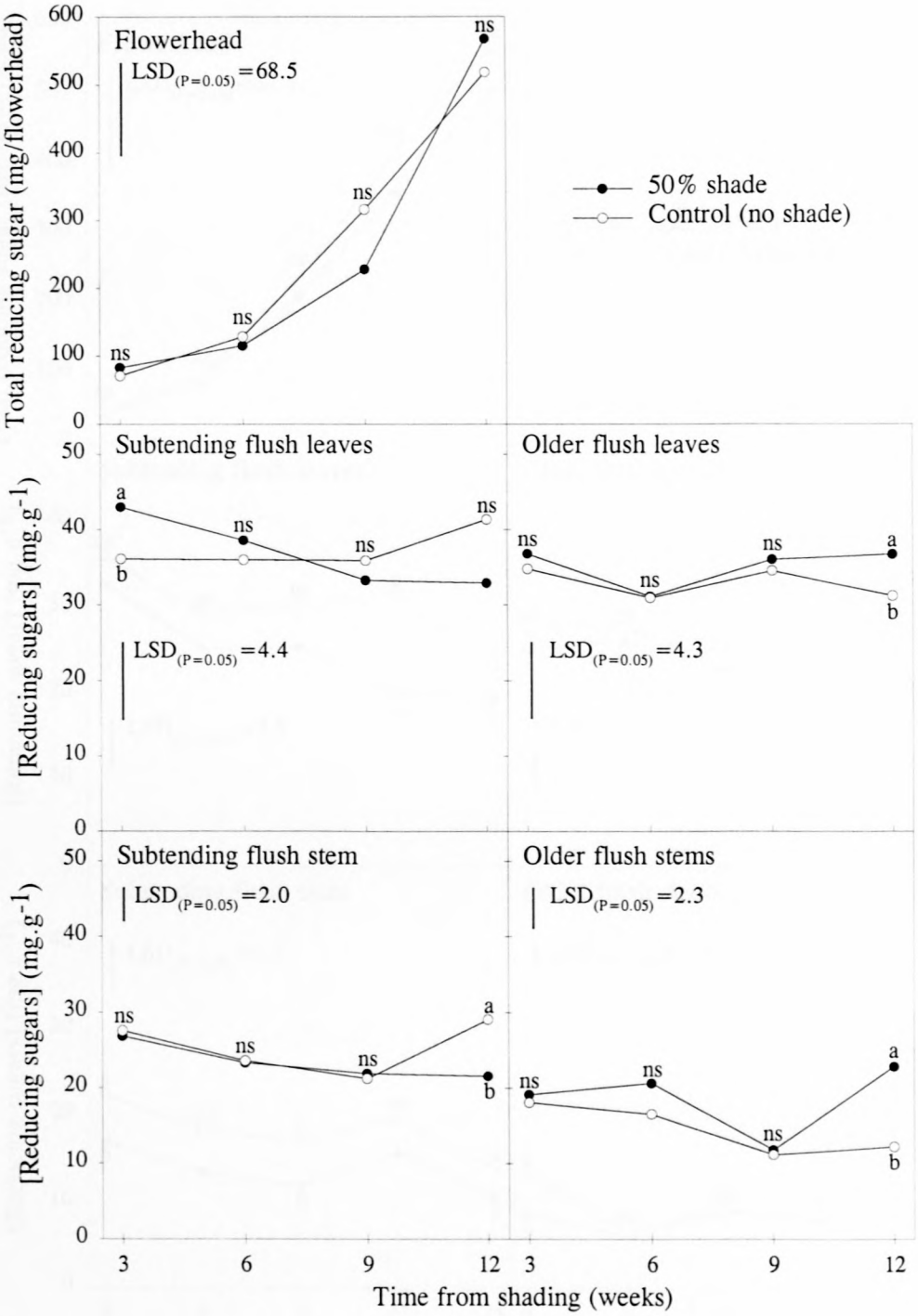
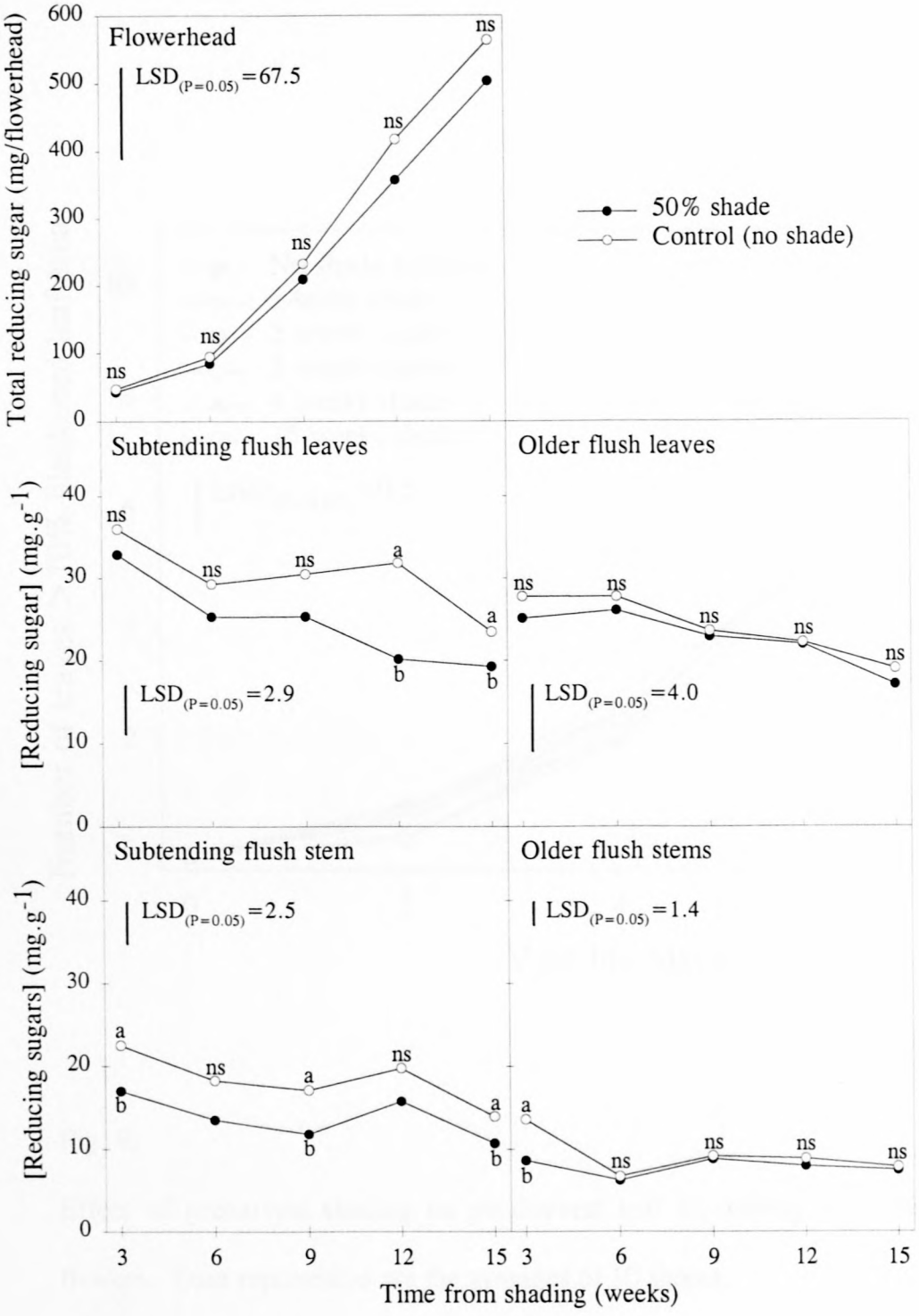


Fig. 7.
Effect of shading (0 and 50%) on reducing sugar content of four flush flowering shoots during flowerhead development. Data represented are the average of 5 shoots. Different letters indicate significant differences at $LSD_{(P=0.05)}$ level within individual shading time; ns = no significant difference. Vertical bars represent $LSD_{(P=0.05)}$ values over time.



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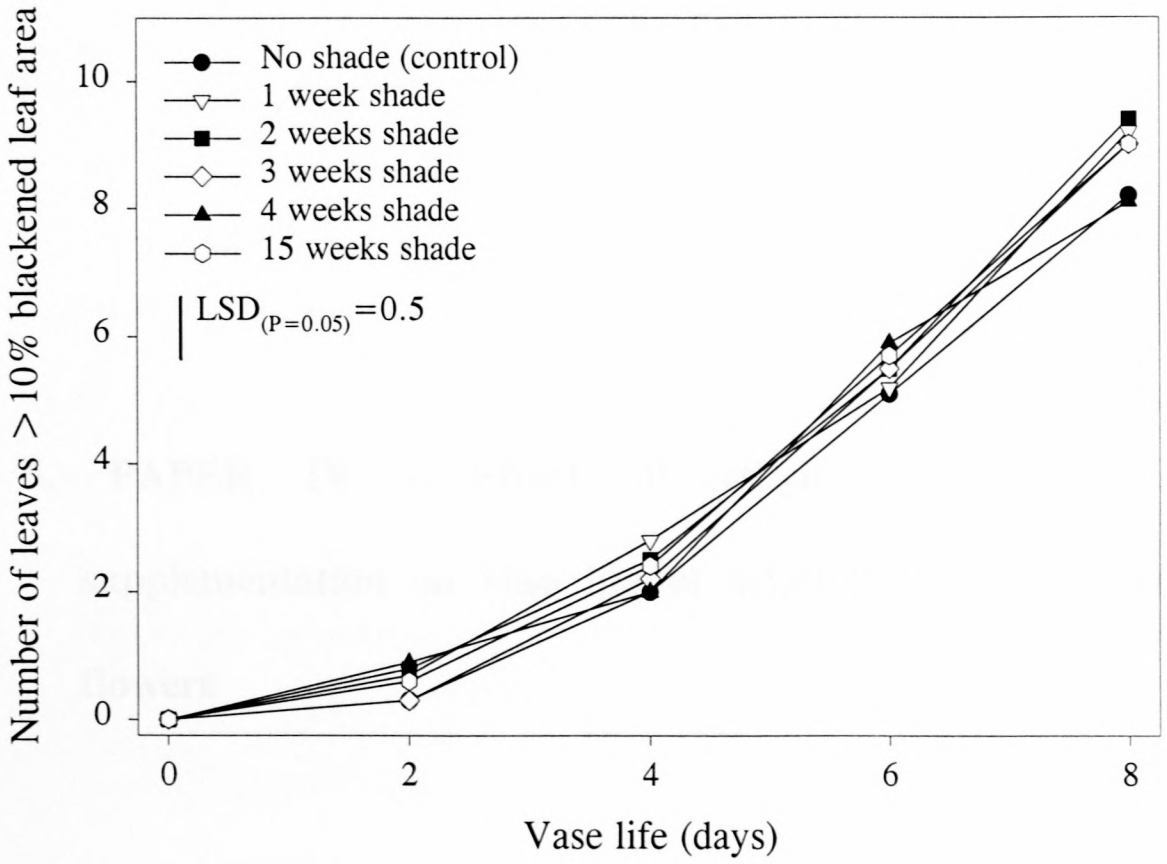

Fig. 9.

Effect of preharvest shading on postharvest leaf blackening of 'Sylvia' protea cut flowers. Data represented are the averages of 10 shoots.

5. PAPER IV - Effect of ethylene and glucose supplementation on vase life of selected Proteaceae cut flowers

Effect of ethylene and glucose supplementation on vase life of selected Proteaceae cut flowers

Vase life of several commercially important *Protea* cultivars is significantly reduced by postharvest leaf blackening. Leaf blackening has been correlated with depleted leaf carbohydrate levels in *P. neriifolia* (McConchie *et al.*, 1991; Jones & Clayton-Greene, 1992; McConchie & Lang, 1993), *P. eximia* (Bieleski *et al.*, 1992) and with increased respiration rates in 'Sylvia' proteas (Paper I). Vase life of *Leucospermum* cut flowers, commonly known as pincushions, is limited by floret collapse and leaf dehydration.

Sucrose pulsing solutions (200 $g \cdot L^{-1}$, 24 h, 1°C) significantly reduced leaf blackening in *P. cynaroides* during long term storage at 1°C (Jones, 1991a) as did sucrose pulsing (200 $g \cdot L^{-1}$, 24 h, 25°C) of *P. neriifolia* during 7 d dark, wet storage at 25°C (McConchie & Lang, 1993). Sucrose supplementation in several other Proteaceae genera has met with mixed success. Holding solutions which incorporated sucrose significantly extended vase life of *Banksia speciosa* (Parvin, 1978), *Banksia prionotes* (Faragher, 1989), *Grevillea* spp. (Lacey, 1982, Faragher, 1989; Vuthapanich *et al.*, 1994; Ligawa *et al.*, 1997), *Leucadendron* cv. Silvan Red (Jones, 1991b), *Leucospermum cordifolium* (Parvin, 1978; Criley *et al.*, 1979), *Leucospermum nutans* and *Leucospermum lineare* (Ireland *et al.*, 1967) and *Serruria florida* (Ireland *et al.*, 1967). However, results for 'Sylvia' proteas indicated that neither holding nor pulsing solutions containing sucrose extended life or reduced leaf blackening (Paper II). The

finding that glucose was beneficial in the extension of both storage period and vase life of 'Sylvia' proteas (Paper II) directed research into its use for other *Protea* and *Leucospermum* cut flowers.

Ethylene has a detrimental effect on the postharvest vase life of many cut flower crops and plays an important role in processes associated with flower senescence (Halevy & Mayak, 1979, 1981). Pre-treatment of *P. eximia* shoots with 4 mM silver thiosulfate did not suppress leaf blackening (Newman *et al.*, 1990; Bieleski *et al.*, 1992). Ethylene evolution was not detected (<0.1 $\mu L \cdot L^{-1}$) in *P. neriifolia* leaves during postharvest assessment (McConchie & Lang, 1993). However, van Doorn (2001) suggested that the presence of fruit, e.g. apples, which are known to generate ethylene during ripening, through postharvest storage and transport may increase *Protea* leaf blackening. Parvin & Leonhardt (1982) reported that silver nitrate (1000 ppm) extended the vase life of *Leucospermum* cv. Hawaii Gold (*L. conocarpodendron* × *L. cuneiforme*) but was of no beneficial in *L. cordifolium* cultivars. Exposure of different *Protea* and *Leucospermum* selections to ethylene was done to determine the role of ethylene in vase life termination.

Materials and Methods

PLANT MATERIAL. Flower-bearing shoots of several *Protea* species and cultivars namely, 'Barbigera' (*P. magnifica*), 'Brenda' (*P. compacta* × *P. burchelli*), 'Cardinal' (*P. eximia* × *P. susannae*), 'Carnival' (*P. compacta* × *P. neriifolia*), 'King' (*P. cynaroides*), 'Pink Ice' (*P. compacta* × *P. susannae*), 'Susara' (*P. magnifica* × *P.*

susannae) and 'Sylvia' (*P. eximia* × *P. susannae*) were brought to the laboratory. Stems were recut to 50 cm and the bottom 20 cm stripped of leaves.

Flower-bearing shoots of six *Leucospermum* cultivars namely, 'Cordi' (*L. cordifolium*), 'Gold Dust' (*L. cordifolium*), 'High Gold' (*L. cordifolium* cv. Yellow Bird × *L. patersonii*), 'Scarlet Ribbon' (*L. glabrum* × *L. tottum*), 'Succession' (*L. lineare* × *L. cordifolium*) and 'Tango' (*L. lineare* cv. Diadem × *L. glabrum* cv. Helderfontein) were used. On arrival at the laboratory stems were recut to 40 cm and the bottom 20 cm stripped of leaves.

All flower-bearing shoots were harvested from commercial plantations near Stellenbosch, Western Cape, South Africa (lat. 33°54'S; long. 18°50'E). The area is classified as having a Mediterranean climate with hot dry summers and an annual rainfall of 600-700 mm falling mainly in winter.

GLUCOSE PULSING OF *PROTEA*. Shoots were placed in 5 L buckets and pulsed with either 0, 1, 2, 3, 4, 5 or 10% glucose at 18±1°C for 24 h. Ten single shoot replicates per treatment were used. After pulsing, shoots were placed into individual vases containing tap water and vase life was assessed in a temperature controlled room (18±1°C) subject to natural light. The number of leaves with at least 10% blackened leaf area was determined daily. Unless mentioned otherwise, involucral bract browning and collapse were not used as a criterion for vase life termination since leaf blackening occurred first.

GLUCOSE HOLDING SOLUTIONS FOR *LEUCOSPERMUM*. Shoots were placed into individual vases containing 0, 1, 2 or 5% glucose, held continuously in those solutions

and vase life assessed for 24 d. Ten single shoot replications per treatment were used. Phytotoxicity, floret collapse, floret whorl development and leaf dehydration were used as criteria in vase life assessment of *Leucospermum* cultivars.

GLUCOSE PULSING SOLUTIONS FOR *LEUCOSPERMUM*. Shoots were placed in cartons stored at 1°C for 3 d. Upon storage removal stems were recut then placed in individual vases containing either 0 or 2% glucose solution for 20 h at 18±1°C (pulsing) before being transferred to individual vases containing tap water. Vase life was assessed as described for 24 d. Ten single shoot replicates per treatment were used for all cultivars, except 'Succession' where 8 single shoot replicates per treatment were used.

ETHYLENE. Flower-bearing shoots of 'Barbigera', 'Brenda', 'Carnival' and 'King' proteas and 'Cordi', 'High Gold' and 'Succession' pincushions (*Leucospermum*) were used. Shoots were placed in buckets containing tap water and cooled inside rooms maintained at 1°C for 6 h. Shoots were then placed in containers attached to a continuous flow of humidified air with 50 $\mu L \cdot L^{-1}$ ethylene or humidified air without ethylene at a rate of 300 $mL \cdot min^{-1}$ using flow boards (Morris, 1969) and needle valves. After 3 d storage (1°C) shoots were removed from containers and recut to 45 cm prior to placement in individual vases containing tap water. Vase life was assessed in a temperature controlled room (18±1°C) for 10 d. Number of leaves with at least 10% blackened leaf area, involucral bract browning and collapse were used as criteria for *Protea* vaselife termination. Involucral bract browning ≥20% and involucral bract collapse ≥10% was deemed unacceptable. Floret collapse was used as criteria for vase

life termination in pincushions. Four replications of six shoots per treatment were used.

STATISTICAL ANALYSIS. Standard analysis of variance was performed using the General Linear Model generated by the SAS® program (Statistical Analysis Systems Institute, 1996). Students t-LSD were calculated at a 5% significance level to compare treatment means.

Results

GLUCOSE PULSING OF *PROTEA*. Increasing glucose concentration was associated with a significant improvement in the vase life of *Protea* cultivars (Table 1). Glucose pulsing solutions had no significant effect on 'Cardinal' leaf blackening reduction, or on vase life extension of 'King' protea flowers. Phytotoxicity was observed in all proteas pulsed with 10% glucose solution, except 'Susara'. In the case of 'Pink Ice', phytotoxicity was observed with glucose solutions of 4% and above.

GLUCOSE HOLDING SOLUTIONS FOR *LEUCOSPERMUM*. Vase life was significantly extended in 'Cordi', 'Gold Dust', 'High Gold' and 'Succession' pincushions held in 1 and 2% glucose holding solutions (Table 2). Phytotoxicity was observed in leaves of shoots held in 5% glucose holding solution. In cultivars responding positively to glucose, floret whorl development was significantly greater in shoots held in 2% glucose solution (data not shown). No significant effect was found when using glucose in holding solutions for either 'Scarlet Ribbon' or 'Tango' pincushions.

GLUCOSE PULSING SOLUTIONS FOR *LEUCOSPERMUM*. There was a significant increase in vase life of 'Cordi', 'Gold Dust', 'High Gold' and 'Succession' pincushions stored at 1°C for 3 d, followed by a 2% glucose pulse (Table 3). Vase life of control shoots, cooled and stored for 3 d at 1°C was significantly longer than control shoots placed directly in water after harvest. No significant improvement in 'Scarlet Ribbon' or 'Tango' pincushion vase life was found with 2% glucose pulsing solution where 'Tango' pincushion vase life was reduced.

ETHYLENE. No significant difference in vase life between flowers treated with ethylene or air was found (Table 4).

Discussion

Significant differences exist in the response of various *Protea* cultivars to glucose supplementation. Four of the six cultivars investigated, namely 'Brenda', 'Carnival', 'Pink Ice' and 'Susara', responded positively to glucose supplementation. It is possible that 'Cardinal' and 'King' proteas utilise sugar(s) other than glucose or that the number of glucose transporters is limited precluding uptake and vase life extension. The significant difference in sensitivity to glucose concentration in 'Pink Ice' (phytotoxic at ≥4%) and 'Susara' (no apparent toxicity), in conjunction with the lack of response in 'Cardinal', a hybrid from the same parents as 'Sylvia' (*P. eximia* × *P. susannae*) indicates the need to direct future research to individual cultivars.

Differences in the response to glucose supplementation were found between pincushion cultivars. Vase life of four of the six cultivars was extended by glucose

holding or pulsing solutions. Differences in carbohydrate utilisation are likely as sucrose supplementation has been found beneficial in extension of *L. cordifolium* vase life (Parvin, 1978). In glucose responsive pincushions there was a significant increase in vase life of control flowers that were cooled and then stored for 3 days at 1°C. This may be attributed to the removal of field heat, consequent suppression of respiration and conservation of carbohydrate reserves, and again emphasises the importance of temperature management for vase life extension.

Exposure to exogenous ethylene did not result in increased leaf blackening or shorter vase life (a specific flower response) in any of the Proteaceae evaluated. This is in agreement with earlier work that STS treatments did not suppress leaf blackening in *P. eximia* (Newman *et al.*, 1990; Bieleski *et al*, 1992) and the report that silver nitrate had no benefit on *L. cordifolium* cultivars (Parvin & Leonhardt, 1982).

Further research on carbohydrate source, concentration, pulsing conditions and storage temperatures, in order to extend vase life of cut Proteaceae flowers, is required.

Literature cited

Bieleski, R.L., Ripperda, J., Newman, J.P. & Reid, M.S., 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117, 124-127.

Criley, R.A., Williamson, C. & Masutani, R., 1979. Vase life determinations for *Leucospermum cordifolium*. Flor Rev. 163, 37-38, 80-82.

Faragher, J.D., 1989. A review of research on postharvest physiology and horticulture of Australian native flowers. Acta Hort. 261, 249-256.

Halevy, A.H. & Mayak, S., 1979. Senescence and postharvest physiology of cut flowers. Part 1. Hort. Rev. 1, 204-236.

Halevy, A.H. & Mayak, S., 1981. Senescence and postharvest physiology of cut flowers. Part 2. Hort. Rev. 3, 59-143.

Ireland, J.P., Meynhardt, J.T. & Strauss, 1967. When proteas become sailors: treatment before shipping. Farming in South Africa 43, 33-35.

Jones, R.B., 1991a. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. Proc. Intl Protea Assoc. 6th Biennial Conf., Perth, Western Australia. pp 313-322.

Jones, R.B., 1991b. A pre-storage sucrose pulse protects *Leucadendron* var. 'Silvan Red' during long term dry storage at 1°C. Acta Hort. 298, 247-253.

Jones, R.B. & Clayton-Greene, K.A., 1992. The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia* R. Br. leaves. Scientia Hort. 50, 137-145.

Lacey, S., 1982. Vase life of *Grevillea* Sandra Gordon. Proc. Production and Marketing of Australian Wildflowers for Export Conf., 1982. Univ. of Western Australia, Perth, Australia. pp 111-113.

Ligawa, J.K., Joyce, D.C. & Hetherington, S.E., 1997. Exogenously supplied sucrose improves the postharvest quality of *Grevillea* 'Sylvia' inflorescences. Aust. J. Exp. Agric. 37, 809-816.

McConchie, R. & Lang, N.S., 1993. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118, 355-361.

McConchie, R., Lang, N.S. &Gross, K.C., 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116, 1019-1024.

Morris, L.L., 1969. A two stage, flow-through system for vegetables. Horticultural Report No. 9, Michigan State Univ., East Lansing, MI. pp 13-16.

Newman, J.P., van Doorn, W. & Reid, M.S., 1990. Carbohydrate stress causes leaf blackening in Proteas. Acta Hort. 264, 103-108.

Parvin, P.E., 1978. The influence of preservative solutions on bud opening and vase life of selected Proteaceae. Proc. First Ann. Orn. Sem., Univ. of Hawaii CES Misc. Publ. 173, 29-33.

Parvin, P.E. & Leonhardt, K.W., 1982. Care and handling of cut protea flowers. Proc. 8th and 9th Ann. Protea Workshop. Univ. of Hawaii, HITAHR Res. Ext. Ser. 018, 20-23

Statistical Analysis Systems Institute, 1996. SAS® User's guide, Version 6.12, Cary, NC.

Van Doorn, W.G., 2001. Leaf blackening in Protea flowers: recent developments. Acta Hort. 545, 197-204.

Vuthapanich, S., Simons, S. & Turnball, L.V., 1994. Effects of harvest maturity and postharvest treatments on vase life of *Grevillea* cv. 'Majestic' inflorescences.

Proc. Australian Postharvest Conf. 1993. Univ. of Queensland, Gatton College, Gatton. pp 45-51.

Tables

Table 1.

Effect of glucose pulsing solution (%) on vase life of selected *Protea* cultivar cut flowers. Data presented are the cultivar means of 10 shoots.

	Vase life (days)						
Cultivar	0% (control)	1%	2%	3%	4%	5%	10%
'Brenda'[1]	5	6	5	7	8	9	1[2]
'Cardinal'[3]	4	4	4	3	4	3	1[2]
'Carnival'[1]	6	6	7	7	8	10	1[2]
'King'[3]	12	12	13	12	14	14	1[2]
'Pink Ice'[1]	5	6	8	10	1[2]	1[2]	1[2]
'Susara'[1]	6	6	7	8	9	12	14

[1] quadratic relationship between glucose concentration and vase life

[2] toxic

[3] no effect.

Table 2.

Effect of glucose holding solution on vase life of selected *Leucospermum* cultivar cut flowers. Data presented are the cultivar means for 10 shoots.

	Vase life (days)			
Cultivar	0% glucose (control)	1% glucose	2% glucose	5% glucose
'Cordi'[1]	9	18	19	4[2]
'Gold Dust'[1]	10	24	24	3[2]
'High Gold'[1]	10	14	15	4[2]
'Scarlet Ribbon'[3]	16	16	16	15
'Succession'[1]	8	16	16	4[2]
'Tango'[3]	20	19	20	20

[1] quadratic relationship between glucose concentration (0, 1 and 2%) and vase life

[2] toxic

[3] no effect.

Table 3.

Effect of glucose pulsing solution on mean vase life of selected *Leucospermum* cultivar cut flowers.

Cultivar	Vase life (days)	
	Control (water)	2% glucose pulse
'Cordi'	12[b]	17[a]
'Gold Dust'	15[b]	24[a]
'High Gold'	12[b]	14[a]
'Scarlet Ribbon'	16[a]	16[a]
'Succession'	10[b]	15[a]
'Tango'	20[a]	18[b]

Cultivar means (n=10: except 'Succession' n=8) within rows followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

Table 4.

Effect of ethylene on post-storage vase life of selected Proteaceae cut flowers. Data presented are the cultivar means of 24 shoots.

Cultivar	Vase life (days)	
	Air	Ethylene
Protea		
'Barbigera'	10[a]	10[a]
'Brenda'	4[a]	5[a]
'Carnival'	10[a]	10[a]
'King'	9[a]	8[a]
'Sylvia'	5[a]	5[a]
Leucospermum		
'Cordi'	9[a]	9[a]
'High Gold'	10[a]	10[a]
'Succession'	10[a]	10[a]

Means within a row followed by superscripts with the same letter are not significantly different at $LSD_{(P=0.05)}$ level.

6. GENERAL CONCLUSIONS.

General Conclusions

Leaf blackening is a serious postharvest disorder affecting several commercial *Protea* cut flower crops grown in South Africa. The negative impact on appearance and profitability, combined with increasing transport costs threaten the South African protea industry.

Low temperatures during handling and storage are essential for prolonging both storage and vase life of 'Sylvia' protea cut flowers. Increasing storage temperatures resulted in higher respiration rates, lower carbohydrate levels and increased leaf blackening in 'Sylvia' proteas. Post harvest removal of field heat should be done as soon as possible in order to minimise carbohydrate depletion. To this end vacuum cooling to obtain a uniform product temperature is recommended. There is a need to identify optimal storage and transport temperatures for different South African Proteaceae to enable sea freight to Europe. Girdling of 'Sylvia' proteas was beneficial in prolonging storage and vase life in combination with low temperature storage (0°C). Girdling has become a recognised practice employed by South African 'Sylvia' protea growers and its commercial use in other leaf blackening *Protea* cultivars merits investigation. Future *Protea* breeding programs should look to the use and selection of non-blackening proteas, in combination with an ability to withstand sea freight duration, to meet market demands.

'Sylvia' proteas did not exhibit a climacteric peak in respiration. Exposure to ethylene did not result in increased leaf blackening or have a detrimental effect on vase life of Proteaceae evaluated.

Glucose supplementation in holding and pulsing solutions significantly suppressed leaf blackening of 'Sylvia' proteas. Furthermore, glucose pulsing solutions enabled the successful three week sea transport of 'Sylvia' proteas to European markets and has become a recognised commercial practice in the South Africa industry and has significantly improved the profitability of the South African protea industry.

At present it is uncertain as to why supplying glucose, instead of sucrose, in both pulsing and holding solutions improved 'Sylvia' protea vase life. Investigation of invertase and sucrose synthase activity may elucidate the lack of response to sucrose supplementation in 'Sylvia' proteas. The varied response to glucose supplementation in the Proteaceae investigated indicates a need to further evaluate different sugars and concentrations supplied at a cultivar level.

The fact that shading did not enhance leaf blackening postharvest may be due to the low levels of carbohydrate found in winter and although shading did result in a decrease in carbohydrate content, shoots were already susceptible to leaf blackening. The appearance of preharvest leaf blackening in shoots shaded three and four weeks prior to harvest corresponded to a period of rapid dry mass accumulation. It is thought that shading at this point, concurrent with an increased carbohydrate demand by the developing flowerhead, caused a temporary limitation in photosynthate production and supply and that phenolic-bound carbohydrate was utilised, resulting in liberation of a

reactive phenolic moiety, resulting in preharvest leaf blackening. Investigation of cytokinin and gibberellin activity, in conjunction with the number of shoots per bearer, during spring flush development is recommended to assist in determination of the cause(s) of pre-harvest leaf blackening.